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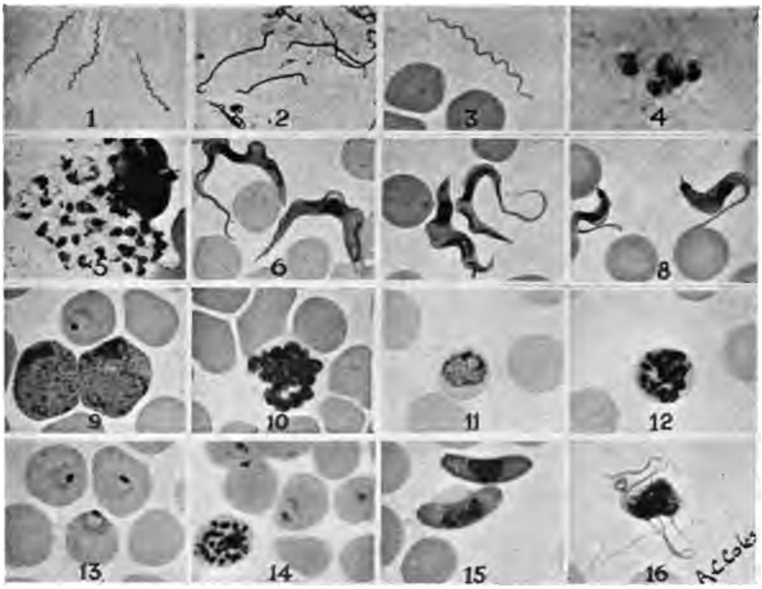

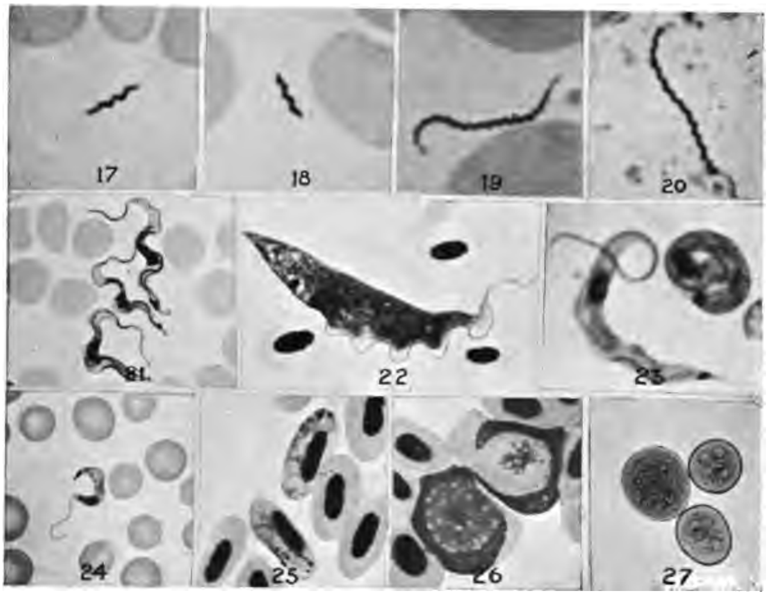

Photomicrographs of Protozoa from Man and Animals.

The sixteen photomicrographs in the upper plate of Protozoa pathogenic to man are taken on a quarter plate at a magnification of 1,000 diameters. (For description see p. 95.)

CRITICAL MICROSCOPY

HOW TO GET THE BEST
OUT OF THE MICROSCOPE

BY

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"CLINICAL DIAGNOSTIC BACTERIOLOGY"

WITH 8 ILLUSTRATIONS

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PREFACE

THIS small book may be regarded as a supplement to, rather than a substitute for, the many excellent works on the microscope now available, and it is written with the hope of assisting microscopists, whatever may be their special line of work, to get the very most out of their instrument.

It does not attempt to describe the various models of the microscope or their accessories, all of which can be seen in the makers' catalogues, and, therefore, illustrations of them are omitted.

My object has been to attempt to answer the question with which I was confronted some years ago:—What method can I adopt or what additional apparatus will enable me to resolve more, and with greater facility?

In the first place I have suggested various slight alterations in the outfit which will materially help one to obtain better results, and in the second place I have described fully the methods and adjustments necessary in order to obtain the highest resolving power of the microscope; but I have also paid considerable attention to the need which is so much felt by members of my profession, especially those who are working at protozoology and tropical medicine, of facilitating the search for a parasite which may be of extremely small size or which may be present in very small numbers. For the latter purpose I have shown the enormous advantage of a dry lens, such as an apochromatic 8-mm. or $\frac{1}{3}$-in. objective, which can be used on covered or uncovered objects by ordinary transmitted or by dark-ground illumination, and I have here reproduced my method of detecting the *Spirochæta pallida* and other spirochætes with this low magnification.

I have also given a short account of photomicrography with a very simple camera, by means of which one is able to take as many as sixteen photographs on a quarter plate.

I owe what little knowledge of microscopy I possess to my friend, Mr. Edward Milles Nelson, who has, mainly by means of letters, rendered me invaluable help during the last twenty years.

Mr. Nelson, for several years President of the Royal Microscopical Society and of the Quekett Microscopical Club, has devoted his life to the optics of the microscope and telescope, and has freely and at all times placed his exceptional knowledge, by communications to the microscopical journals and to the *English Mechanic,* at the disposal of all microscopists.

I have taken the liberty of quoting very fully from his correspondence to me and from his articles in the journals, but I would like expressly to state that Mr. Nelson should not be held responsible for any unscientific statement I may have made.

To my friend Mr. William Harding I am greatly indebted for his constant aid in matters scientific and mechanical.

YORK HOUSE,
BOURNEMOUTH.

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CRITICAL MICROSCOPY

THE MICROSCOPE AND ITS ACCESSORIES.

The Microscope.

It is obviously impossible for me to give a detailed description of the many excellent microscopes now on the market, and I have neither the wish nor the ability to suggest this or that model as being the best. I will content myself with briefly pointing out some of the essential features that are requisite for the highest form of microscopic research.

The English-made microscope of to-day is an instrument of precision, whilst some, like the Powell and Lealand model, have never been equalled in the world. I would, therefore, advise the reader, if he is about to purchase a microscope, to obtain the illustrated catalogues of the various makers, *e.g.*, Watson, Baker, Beck, Swift, etc., and make his selection, remembering that he wants his instrument to be suitable, not only for everyday work, but also for research and even for photomicrography.

When a microscope is chosen, whatever be the immediate use to which it is to be placed, it is advisable to select a stand that is specially adapted to take further apparatus or fitments when the need arises. Many workers imagine that the very highest work can only be done with a complicated and expensive microscope. This, in my opinion, is a mistake. I will mention as an example one model, the " Service " microscope recently introduced by Watson, which embodies the suggestions of the British Science Guild. This instrument in its simplest form is well suited for a beginner, but when fitted with the specially-made mechanical stage and rack and pinion sub-stage, with centring screws, it is capable of doing critical work of the highest order.

As regards the Continental, specially the Zeiss and Leitz, models, they are of great excellence above the stage, but below that level they are, at present, absolutely unfit for any critical microscopic work; but that they may be brought up to the standard and requirements of such by fundamental alterations in their sub-stage I have very certain knowledge.

I have a Leitz stand C with an English centring sub-stage, and fitted with a rackwork for the draw-tube, and I want nothing better.

Before the War, Leitz had put on the market a new model, combining the characteristics of the English and the Continental stands, with a tripod base, a sub-stage with centring screws, and a built-on stage.

Spitta suggests that the absence of a centring arrangement in the sub-stage of the Zeiss microscope can be remedied by "a centring fitting which drops into the sleeve of the condenser, which, though primarily designed for using objectives as condensers (and so, in consequence, supplied with the 'universal fitting,' the same as the nosepiece), will take an English condenser just as well as an objective. Messrs. R. and J. Beck make a similar class of fitting, which we have used for years with great pleasure."

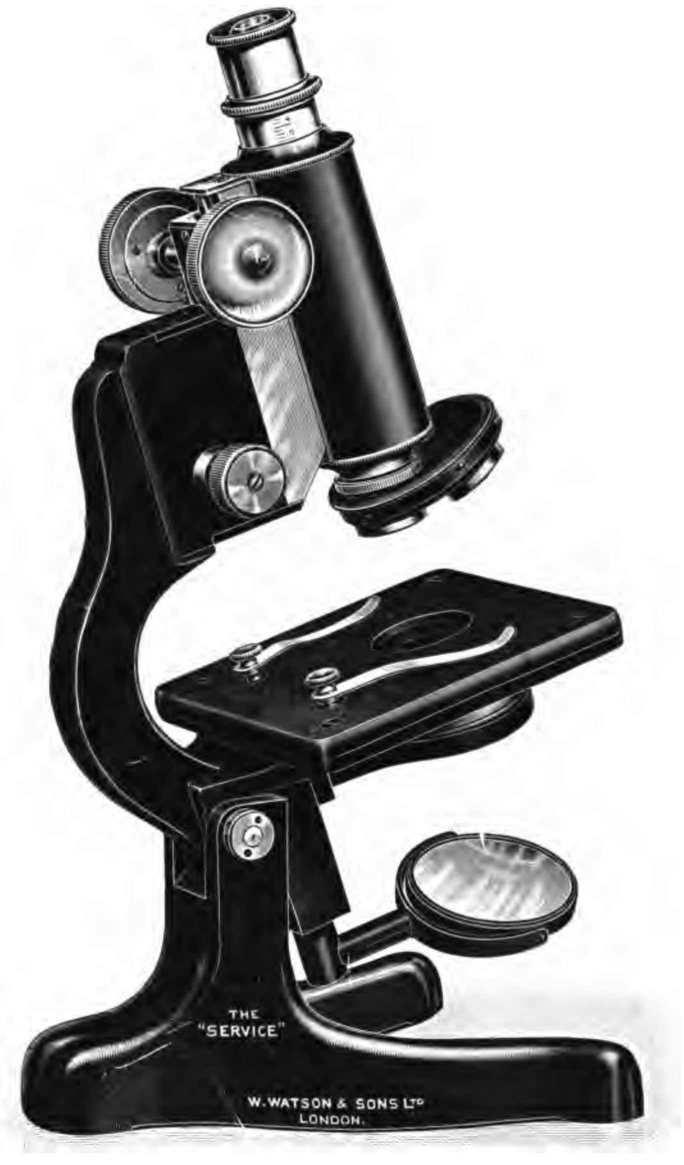
He points out, however, that these adaptors will not take condensers of large size. I have not seen this method of overcoming the difficulty, but, considering that such an arrangement only moves the condenser, and not the iris diaphragm or the stop carrier, I fail to see how one can either centre the condenser by means of the iris or use dark stops.

I want briefly to point out to those who already possess, as well as those about to purchase, a microscope certain defects which are inherent in many instruments, and which require correcting before work of a critical nature can be undertaken.

The stand should have the maximum of steadiness with the minimum of weight, and it should remain firm when the microscope is placed in the horizontal position. Most Continental and many English models completely fail to fulfil these requirements.

Continental manufacturers are more practised in making horse-shoe feet (which are, by the bye, an English invention) than

PLATE I.



WATSON'S "SERVICE" MICROSCOPE.

(Facing p. 2.)

claw feet. Therefore, in selecting a Continental stand, if there is a choice, choose a horseshoe foot, as it will be found better made and firmer.

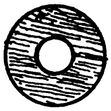
If, however, the microscope be fixed to a board, a plan I have adopted for many years, and which is described later, this defect is easily remedied.

The tube of the body of the microscope is, on some of the higher classes of instruments, made of large diameter, presumably to prevent internal reflection, especially in photomicrographic work.

Personally, I have found no disadvantage whatever in the ordinary narrow tube in photographic work of the most critical nature, provided that a much more important point is attended to, viz., the provision of a suitable stop in the draw-tube.

Mr. Nelson pointed this out to me some years ago. He says: "I had my Leitz lenses before the Leitz microscope came, and I knew they were all right, because I had tested them on my Powell stand. When I put them on the new microscope, they were all wrong. The images were all hazy, milky, foggy, etc. I soon guessed what was wrong: the microscope draw-tube had no stop in it, and, so far as I know, none of the Continental tubes has! The stop at the end of the draw-tube is ornamental, but of no use for stopping reflections from the tube; its only use is to prevent the draw-tube being pulled out. The compensating oculars of high powers have their diaphragm outside the eyepiece, and this will take some of the reflections off, but the ordinary eyepieces take most of them in.

"If you examine the Ramsden disc at the top of the eye-lens, you will find it like this:



The large circle is the image of the back lens of the objective, the small central circle that of the stop at the back of the sub-stage condenser, and the shaded portion is a weaker kind of light which is the reflection from the inside of the microscope tube. This, of course, ought not to be there at all. The annulus, shaded in the Figure, should be as black as night.

"I soon put matters right by taking an impress of the top of the microscope draw-tube on a piece of card, finding accurately the centre of this disc, and then drawing a circle 14 mm. in diameter and cutting it out with a pocket knife, and then cutting the annulus out of the postcard very accurately to the mark of the tube. This was pushed down the draw-tube with the longest eyepiece about 2 in., and I pushed it down another ½ in. where there was no risk of its interfering with the field. The annulus in the figure above is now quite black, and the fog and haze are all gone.

"How many Continental microscopes, as well as their English and American copies, are suffering at this present moment from fog and haze disease? In photography, of course, no light is wanted in the camera except the image-forming rays which pass through the Ramsden disc. The reflected light which passes through the eyepiece on the outside of the Ramsden disc when the stop is not in the tube is not very strong, but when the exposure is long it has its effect in fogging plates. This is important, because want of contrast is the photomicrographer's chief difficulty."

The draw-tube may with advantage be fitted with a rack and pinion movement, but this is not essential, provided the tube works perfectly smoothly. Much damage may be done with a draw-tube that works very stiffly, but this is easily corrected by taking the tube out and polishing it thoroughly. The divisions on the tube do not include the thickness of the nosepiece, so that 15 to 20 mm. must be added.

The coarse adjustment should, on the other hand, not work too easily. I found, when I first had my Leitz, that, when the Abbe Camera Lucida was in position, the focus kept altering; this was at once corrected by tightening two vertical screws placed just above the shoulder of the instrument.

The fine adjustment should be a very slow movement, especially for photomicrography. In the Berger adjustment of Zeiss there are five turns of the screw to 1 mm. Leitz makes two forms. In the one I have there are ten turns, and the other five turns, to 1 mm.

The Powell and Lealand has fifteen turns to the millimetre, and yet Mr. Nelson says he finds this too quick and would gladly have it altered to thirty.

The stage should have a central aperture of not less than 1½ in., better 2 in. in diameter, otherwise when an oil immersion condenser is used, the oil gets on to the surface of the stage. I have recently seen a very fine English instrument with the central hole about ¾ in.!

A rotating stage is, as far as I am concerned, perfectly useless, except that it is a little easier to clean.

Every microscope must have a **mechanical stage,** which should preferably be an intrinsic part of the stage. In the best English forms the slide does not move over the surface, but the whole stage moves in both directions. These have the advantage that the oil from the oil immersion condenser rarely touches the stage, and the slide does not rise from the surface as it is apt to do in many of the detachable stages. Generally, however, we have to be satisfied with some form of detachable mechanical stage, but in any case it is essential that there should be at least a movement of 2 in. in the horizontal and one in the vertical direction, and there should be divisions reading by verniers so that the exact position of an object may be noted.

A matter requiring more attention on the part of the makers is the best position of the divisions; in many cases they are very difficult to read.

The sub-stage is one of the most important parts of the modern microscope, as so much depends upon the fitment of the sub-stage condenser. It should be provided with a rack and pinion focussing adjustment, and have centring screws by means of which the condenser can be placed exactly central with the objective. Centring screws are lacking in all or nearly all of the Continental instruments, and I have seen two English models in which, although centring screws were provided, it was impossible, owing to faulty workmanship, to bring the condenser to a central position.

A fine adjustment to the sub-stage would be of advantage in some work, especially with dark ground illumination. The sub-stage should maintain its central position when it is racked up and down.

Some sub-stages have a swing-out arrangement for turning the condenser and its fittings out of the optical axis. This is a slight help in changing condensers, but unless it is well made it tends to make the whole of the sub-stage unsteady, and it may prove a real disadvantage. The condenser and its mount may be placed in the sleeve of the sub-stage from below or from above.

The former is sometimes spoken of as an under-stage and the latter as a sub-stage mount. There is probably very little preference, but in the sub-stage mount, *i.e.*, fitting in from above, the iris diaphragm and the stop carrier are nearer the back lens of the condenser.

A very great convenience is to have an additional stop carrier fitted below the ordinary stop carrier, and made to revolve in from the opposite side. I have such an arrangement on my under-stage fitment in order to carry a colour screen.

The mirror is by no means such an unimportant part of the microscopic outfit as the text-books would lead one to suppose. I have repeatedly met with the following defects, either of which would, in my opinion, preclude critical work.

1. It may be found that, owing to imperfections of the surface or because it is wedge-shaped, the mirror gives several reflections of the edge of the flame at the same time. This is of common occurrence and renders critical work, especially with dark ground illumination, impossible.

It may be removed by first placing the lamp in one definite position, and then rotating the mirror in its cell till a spot is reached at which the images will all be superimposed.

It is not always easy to twist the mirror round in its setting, but by gentle pressure, and reversing the direction occasionally, the object may usually be attained.

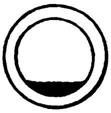
2. The mirror should be attached to the tail piece in such a manner that its centre is in the optic axis, and yet will allow the mirror to be turned aside or easily removed if it is desired to dispense with it and use direct light. Where the tail piece of the microscope is square, the mirror can be moved up and down it,

or can be slipped off, but when it is round it is necessary to have some clip or spring to show when the mirror is in its right axis.

3. Another and, in my experience, a very common and more important defect is that in which the stem holding the mirror to the tail piece is too long. If a line were drawn through the centre of the draw-tube and main-tube of the microscope, it should strike the face of the flat surface of the mirror, and not the space on the brass mount intermediate between the curved and flat mirror.

Or, as Mr. Nelson suggests, remove all lenses from the body and the sub-stage, extend the draw-tube and look down it at the mirror. The central line of vision should strike the centre of the mirror and not the space on the brass mount.

To find whether or no this defect is present, and at the same time to see its effects, incline the microscope, say, to an angle of 45 degrees, place a slide on the stage and focus the object and the image of the flame with the $\frac{1}{12}$-in. objective. Examine the back of the objective by removing the eyepiece and looking down the tube, or better still, keeping the ocular in position, examine the image in the Ramsden disc by means of another eyepiece held inverted over the ocular in use. If there is a dark area at the lower part of the back of the lens, thus:



the stem of the mirror is too long. The more the microscope is inclined the more marked the result, and when not seen with a dry condenser, it may be made evident by using an oil immersion condenser.

Another test may be very simply tried. With the microscope in the same position and an eyepiece in the tube, hold a long needle or a piece of wire transversely across the surface of the mirror, and get the image of this in the centre of the field. This line should be approximately the centre of the mirror; if it is found to correspond to the top edge of the mirror, the stem of the mirror is too long, and a piece should be taken off it.

Mr. Nelson says: "The mirror error you mention is well known. I have seen an object on the stage coloured yellow by reflection from the lacquer on the upper edge of the mirror cell! "

The mirror in the Continental microscopes is apt to be too small in diameter. The mirror in my Leitz microscope has a diameter of 1·75 in. and an area, therefore, of 2·4, whilst Mr. Nelson says, " that the mirror in his Powell and Lealand instrument has a diameter of 3 in. and an area of 9 in., or 3·6 times as much. Even with this large mirror one has to be on the watch; how much more then with a small Continental mirror is it necessary? "

It may, in many cases at least, be temporarily remedied by inclining the microscope less, by raising the lamp higher or, possibly, by sliding the mirror down the tailpiece, but neither of these may be convenient and, until it is permanently removed, no critical work can be undertaken. Messrs. Watson have recently made a new mirror of stainless steel. I have had an opportunity of examining this and find that it gives a perfect image of the edge of the flame without any secondary reflections, and as the mirror is quite thin its use would entirely obviate the first and third defects, just mentioned, which are so commonly found in the ordinary mirror.

Mr. Nelson has made some interesting comments on the mirror. He says: " There is no possible advantage of a right-angled prism (costing £25) over a mirror. I cannot say I can mention any definite object or object glass in which I could perceive any difference with the mirror or with the lamp direct. There is no perceptible difference in the critical nature of an image by direct or mirror illumination, there is less light in the latter case. It matters little whether the mirror be dusty or scratched, or the silver in bad condition, the only effect is a little less light, which is extremely unimportant. An old scratched mirror will yield as critical an image as the finest worked up silver on glass of a Newtonian flat."

The Objectives.

The ordinary pathological or bacteriological outfit generally includes the following achromatic objectives:—1-in. or ⅔-in.

N.A. 0·2 to 0·26, ⅙-in. N.A. 0·65 to 0·85, and $\frac{1}{12}$-in. oil immersion of N.A. 1·3, and, in the case of those doing research work or possessing more means, apochromatics, 16 mm. ⅔-in. N.A. 0·3, 4 mm. ⅙-in. N.A. 0·95, and the 3 mm. ⅛-in. or 2 mm. $\frac{1}{12}$-in. of N.A. 1·3 to 1·4.

The question may be asked, Do you consider either of these the best combination of lenses to have for all-round daily use, for dark-ground illumination, for the highest critical work, and for photomicrography? My answer would depend largely upon the nature of the work and the extent of the means available.

For such work as pathology, bacteriology, and protozoology, I should certainly say that the above is not the best combination.

I possess the following objectives:—Leitz achromatic ⅔-in. N.A. 0·3,* Zeiss and Leitz apo. 8 mm. ⅓-in. N.A. 0·65, and Leitz achromatic oil immersion $\frac{1}{10}$-in., $\frac{1}{12}$-in., and fluorite $\frac{1}{16}$-in. N.A. 1·3 to 1·32.

I have on the microscope for constant use the ⅔-in., the 8 mm. and either the $\frac{1}{10}$-in. or $\frac{1}{12}$-in., and reserve the $\frac{1}{16}$-in., a fluorite of N.A. 1·32, an extraordinarily fine lens, for special occasions and for photomicrography. I wish for nothing better.

I have had others, such as Leitz fluorite ⅙ N.A. 0·82, a Zeiss 4 mm. apo. N.A. 0·95, and a Leitz apo. 2 mm. of N.A. 1·40, but these I discarded, as they did not serve my purpose.

I know nothing of the requirements of those working on diatoms or of the *dilettanti*, but from what I have seen of the latter their pleasure seems to consist largely in purchasing, possessing, and playing with lenses of various powers, aperture, and quality, and they probably have never realised all the refinements of modern microscopy, such as critical images, corrections for cover-glass thickness, etc.

For the **low power objective** I should choose a ⅔-in., ¾-in., or 1-in. achromatic, and not an apochromat, unless cost was of no importance. I say this because for our work we do not require a lens that will stand high oculars or be of superb quality, as it will often be used for uncritical observations.

---

* Nelson points out "that anything in a ⅔-in. objective over 0.26 N.A. is useless unless it is an apochromatic lens. If an achromatic lens has a greater N.A. than 0.26, and is engraved ⅔, then the engraving is wrong. Leitz 3 is a ½ in.

"Leitz 3a is a semi-apo., and is a very fine lens, and only costs 5*s*. more than the No. 3, and it is well worth it."

For the **medium power objective** I would choose the finest apochromatic 8 mm. or ⅓-in. of N.A. 0·65 lens that I could obtain. This lens has the following features, which render it invaluable :—

1. Its N.A. (numerical aperture) is not too high ; therefore, its penetrating power is considerable.

2. Its N.A. is not too large for dark-ground illumination obtained by means of a condenser, used dry, with a stop.

3. Its O.I. (optical index) is very high, 21·7.

4. It gives, with suitable oculars and tube-length, a magnification varying from less than 60 to nearly 600 diameters.

5. It will stand the highest eyepiece, viz., Comp. Oc. 18.

6. It will bear the most searching test that any objective can be put to, viz., dark-ground illumination.

7. It requires very little alteration of the tube-length for a marked variation in the thickness of the cover-glass, and it will work through a thickness of cover that would completely ruin a 4-mm. apo.

8. It will give a critical image of an uncovered object at a tube-length not exceeding that of the microscope in use ; in my case about 210 mm.

9. It has a very long working distance, and there is no fear of its front lens coming in contact with water or oil on a slide.

I have later on described my method of obtaining dark-ground illumination on **stained, but uncovered or unmounted, films,** and I would here merely call attention to the value of the examination of such films by ordinary illumination with the 8-mm. or ⅓-in. objective.

In this method it is seldom necessary to clean a cover-glass and as a matter of fact I only use covers for living preparations or for permanent mounts.

For example, suppose one was about to examine a film of blood for the malarial parasites, I should stain the films with Giemsa or Leishman's stain for, say, fifteen minutes, as described at the end of the book, wash with distilled water, and dry the under-surface of the slide. Then I should place it on the stage, the upper surface being still wet, and examine with the 8-mm. and comp. oc. 6, having pulled the draw-tube out to its fullest. All but the smallest ring parasites would be easily seen with this power. If I were in doubt, I should put in the comp. oc. 18, and examine more carefully any suspicious looking body. If I failed to find any parasites, I should remove the film, dry it, and pour over the surface a drop or two of liquid paraffin, not cedar oil, and let it drain for a second or two. Then, replacing it on the stage, I should examine it more carefully with the same objective and comp. oc. 6 at the same tube-length.

If I were still doubtful about the nature of any minute body, I should turn on the $\frac{1}{12}$-in. objective and oil it to the surface with cedar oil, not liquid paraffin. The extraordinary large field when using the 8-mm. lens is of the very greatest help in the hunt for a parasite.

Cannot the apo. 4-mm. or $\frac{1}{6}$-in. of N.A. 0·95 or a $\frac{1}{6}$-in. achromatic of N.A. 0·7 be used in the same way? No, certainly not. Its N.A. is too high for dark-ground illumination; it cannot give such a low magnification; it requires very marked alteration for slight variations in the thickness of the cover-glass; it has a very short working distance, and oil or water on the film may easily touch the front lens; and lastly, it cannot be used on an uncovered object.

I have recently found, by experiment, that an excellent Leitz $\frac{1}{6}$-in. of N.A. 0·82, which normally required a tube-length of 170 mm. on a mounted object, required a tube-length of 880 mm., or about 35 in., when used on an uncovered object!

Can nothing be done to an apo. 4 mm. of N.A. 0·95 or an achromatic $\frac{1}{6}$-in. of N.A. 0·8, in order to make it possible to work by ordinary illumination or by dark-ground illumination obtained by a dry condenser on an uncovered object?

The Leitz $\frac{1}{6}$-in. can be stopped down by a funnel or some such means so that its aperture is 0·65; it will then give a good dark ground with a dry condenser on a mounted object, but not on an unmounted film. Some forms of $\frac{1}{6}$-in. objectives in which the lenses can be separated can be adapted for an uncovered object by unscrewing the front lens. An apo. 4 mm. of N.A. 0·95 can also have a stop fitted to it, cutting the N.A. down to 0·65, and it will then give a dark ground with a condenser used dry on a mounted, but not on an unmounted, object.

Some years ago I put the problem of the possibility of adapting the 4 mm. apo. to uncovered objects to Mr. Nelson. He wrote: "Not having worked at unmounted specimens, I was not aware that the range of the screw-collar of the 4 mm. was insufficient to adjust the lens when used that way.

"Our English lenses had usually lots of range, and could be used for covered or uncovered objects; in fact, the mounts of old lenses were so engraved. But the Zeiss and Winkel lenses are, I noticed, engraved 10 and 20, which, I take it, refer to the thickness of the cover-glass in millimetres. So one of these lenses put at 10 is not right for an uncovered object; but it is a simple matter to put it in adjustment. Thus, unscrew the lower part of the mount below the collar ring and use that for a collar correction; of course, putting the collar ring to 10 in the first instance.

"I have just adjusted my lens in that way for your uncovered slide for a tube-length of 170 mm., although it is a long tube lens.

"Of course, the makers would make the collar to turn further, from 10 to 0, if desired; nothing would be simpler.

"A Leitz 6a with a correction collar would do, but a 4 mm. apo. would be better."

From the above it is evident that the 8 mm. apo. objective is of the very greatest value, both for searching and for all methods of dark-ground illumination; that a $\frac{1}{6}$-in. or a 4 mm. apo. is, in its present condition, of very little use, but that the latter can be rendered serviceable by some structural alterations; but even then it will not have all the advantages of the apo. 8 mm.

For the **high power objective** I would choose an achromatic or fluorite $\frac{1}{10}$-in. or $\frac{1}{12}$-in. oil immersion lens of N.A. 1·3.

I may be considered unorthodox when I state that with my present knowledge I cannot appreciate all the so-called advantages of the apochromatic objectives 3 mm. and 2 mm. of N.A. 1·4. These lenses, which are certainly of the highest order, require great care in their use, owing to the hyperhemispherical form of the front lens, and they are very costly.

Mr. Nelson, in answer to a question of mine many years ago, said: "There is very little difference between a good achromatic $\frac{1}{12}$-in. and an apochromatic $\frac{1}{12}$-in. in visual work when a screen is used. Photographically there is a difference." More recently he has pointed out that, when colour screens and isochromatic plates are used, there is practically no difference in their performance in photomicrographic work.

"*Re* achro- *v.* apochromatic objectives. Photography is a monochromatic affair; therefore, so far as the photographic image, *per se*, is concerned, the correction which brings two or three different coloured rays to one focus is of no importance.

"When an ordinary plate is used, the objective must have its chemical and visual foci coincident, for the purposes of focussing; but Mr. T. F. Smith showed, in 1893, that, when an isochromatic plate was used, an achromatic objective would take as good a photograph as an apochromat, even when no coloured screen was used. The plate selected its own ray, which was similar to that which the eye had focussed! If an ordinary plate were used, the image with the achromat was all fluff and quite indistinct; but with an apochromat and an ordinary plate the sharpness of the image was restored. Nevertheless, with an isochromatic plate the image was improved, even with an apochromatic objective."—*Nelson.*

Personally, I have never seen an apo. 2 mm. which gave me an appreciably better image than a good achromatic or fluorite lens, and I have examined a fair number of apochromatic objectives of N.A. 1·4.

When one sees workers using a 2 mm. apo. of N.A. 1·4 in conjunction with a common Abbe condenser which can only give a totally inadequate cone, one realises that they would have obtained infinitely better results by using a good achromatic $\frac{1}{12}$-in. objective in combination with a suitable achromatic substage condenser, and at very considerably less cost.

The Optical Index of Objectives.—Dallinger, writing on "The Microscope," eighth edition, says: "In the Zeiss apochromatic series of objectives the 24 mm. of 0·3 N.A. and the 12 mm. of 0·65 N.A. (both for the long tube) may be considered as lenses of the very highest order, the relation of their aperture to their power is such that everything which a keen and trained eye is capable of taking cognisance of is resolved when the objective is yielding a magnification of twelve times its initial power; for this purpose an objective must have 0·26 N.A. for each hundred of combined magnification. Under these conditions, an object is seen in the most perfect manner possible.

" In this connection Mr. Nelson has suggested (*Journal of the Royal Mic. Soc.*, 1893, p. 12) that the term optical index should be added to that of numerical aperture.

" The optical index or O.I. is the ratio of the numerical aperture (× 1,000) to the initial magnifying power. Thus, the numerical aperature of the Zeiss apo. 24 mm. is 0·3, and the initial power is 10. Then its O.I. is $\frac{300}{10} = 30$.

" The O.I. of the 12 mm. apo. of 0·65 N.A. is $\frac{650}{21} = 31$.

" That of the $\frac{1}{8}$-in. homogeneous immersion of 1·4 N.A. is $\frac{1{,}400}{83} = 17$.

" Compare now these figures with an old water immersion $\frac{1}{50}$-in. of 1·1 N.A. $\frac{1{,}100}{550} = 2$. The value of these figures will be apparent when we remember that any lens used with a 10-power eyepiece must have an O.I. of 26, to resolve all detail visible to a keen eye.

" The optical index, therefore, tells us that the $\frac{1}{50}$-in. water immersion of 1·1 N.A. had a vast amount of empty magnifying power, while on the other hand, the 24 and 12 mm. will both stand a higher power eyepiece than 10, nay, even require it before the detail resolved by them is made visible to the eye. It also shows that the $\frac{1}{8}$ of 1·4 N.A. will stand a higher eyepiece without arriving at an empty magnifying power than the $\frac{1}{12}$ of 1·4 N.A., where O.I. is 11.

" As it is more difficult to put aperture into a lens than power, the O.I. becomes also an index of the money value of a lens."

Mr. Nelson in a recent letter to me has given some fuller details of the above subject, which, although attributed to Dallinger, was written by Mr. Nelson.

"Here is a simple explanation of O.I., 'Optical Index.' It is the N.A. × 1,000 (to get rid of the decimals) and divided by the initial magnifying power of the objective. The initial power is, of course, $\frac{10}{\text{focus}}$.

"Now you know and appreciate the good qualities of your 8 mm. ⅓-in. apo. Why? It has an N.A. of 0·65, so have other lenses, its resolving power is proportional to its N.A., but there is something else, and that something is its O.I. The initial power is 30 and the O·I. $= \frac{650}{30} = 21{\cdot}7$.

"Now suppose you have a 4 mm., a ⅙-in., and you cut a cardboard stop and put it at the back of the lens, and so reduce its aperture to 0·65 N.A., would you like that lens as well as your ⅓-in.?

"Both are apos. with the same N.A., and if with the ⅙-in. you halved the eyepiece about the same power, would you like the result? No, of course you would not. Why? Because of its low O.I. The initial power is 60 and $\frac{0{\cdot}65 \times 1{,}000}{60} = 10{\cdot}8 = \text{O.I.}$

"I have a ⅔-in. objective, its initial power 15, its N.A. 0·30, it is always on the nosepiece. Why? Because its O.I. is 20. I have several other ⅔-in. objectives, all good lenses, but they are seldom used, because they have less optical index."

The Sub-stage Condenser.

The ordinary Abbe sub-stage condenser, so much in general use, is totally unsuited for any critical work. Mr. Nelson tells me that the large two-lens Abbe is a better condenser than the three-lens form.

It is essential that the condenser be achromatic and of a power somewhat near that of the objective in use.

Achromatic condensers are made by Watson, Swift, Beck, Baker, etc. I have used for many years a Swift and a Watson's achromatic sub-stage condenser of about the same power.

The parachromatic condenser of Watson is a $\frac{2}{7}$-in., with a total aperture of 1·0, and an aplanatic aperture of 0·9, and when the top lens is removed, $\frac{4}{10}$ in. in power, which renders it suitable for low powers.

For all-round work and for dark-ground illumination, either of these condensers will give excellent results. They both have the Roy. Mic. Soc. thread and are, therefore, the size of an objective. Another achromatic condenser of somewhat lower power and of larger size is the "Universal" of Watson, which is $\frac{4}{10}$-in. in power, and has an aplanatic cone of 0·95. Baker makes a similar one.

These are very highly spoken of, but I prefer the smaller size and higher power as being, I think, more suitable for use with the $\frac{1}{12}$-in. objective, and somewhat easier to use for dark-ground illumination. When it is necessary to get the very most out of an objective and also to obtain the finest dark-ground illumination, it is necessary to have an oil-immersion sub-stage condenser.

The oil-immersion achromatic condenser was invented by Mr. Nelson. It was adversely criticised by Abbe and others. Abbe said that an aplanatic condenser was useless.

Watson's holoscopic, giving an aplanatic cone of 1·3 to 1·4 N.A. when oiled, is, in my opinion, the best. Beck makes one somewhat like it at a lower price.

The Continental makers have, till recently, only made indifferent condensers, but now they are producing some excellent models. I have an oil-immersion condenser by Leitz, which Mr. Nelson tells me is a very good one.

It is 1-in. in power and has an aplanatic cone of about 1·3 N.A., and a very large optical index of 125. It will, he says, work well with objectives from $\frac{2}{3}$-in. to $\frac{1}{12}$-in., and I find it may be used with somewhat thicker slides than the holoscopic. It is of large size and requires a special fitment. On the whole, I have not found it as convenient as the holoscopic condenser. The Koristka oil-immersion condenser of 1·3 N.A., of the same power as Watson's, is very well corrected.

According to Mr. Nelson, "The proper test for a condenser is made by putting a screen with a 1½ or 2 mm. hole in it, as close as possible to the flame, and by using it as an illuminant. The back of the objective will now appear with a bright central spot, surrounded, at a distance which depends upon the aperture, by a bright annulus. If the condenser be aplanatic, there will be a large unbroken disc. Good sub-stage condensers with large aplanatic apertures are quite rare. Usually condensers if aplanatic have insufficient focal length to work through an ordinary slip, but if they work through a slip they are usually not aplanatic."

An oil immersion condenser cannot be used as a dry condenser if the best results are to be obtained, any more than an oil immersion objective can be used without oil.

Immersion Cedar Oil.—During the War thickened cedar oil for use with oil immersion objectives was difficult to obtain, and the thin cedar oil of commerce was supplied to most of the bacteriological laboratories. The latter has almost the same refractive index as the former, and, according to my experience, it necessitates but a slight increase in the tube length of the microscope in order to obtain perfect results.

The thin cedar oil has, however, one serious disadvantage, and that is it is too thin, and when a drop is placed on the slide it does not retain its spherical form, but immediately runs down the surface, especially when the microscope is much inclined.

The front lens of many an immersion objective has been broken on this account, because when thick cedar oil is used the objective is seen to touch the oil long before it is in actual contact with the surface of the slide, but when thin oil is used, as soon as the lens is seen in contact with the oil it is almost, if not quite, touching the slide, and requires racking up, not down, as in the former case.

I made some immersion oil by dissolving very clear Canada balsam, from which the xylol had evaporated, in thin cedar wood oil, and letting the preparation remain in the incubator until it was of the right consistency.

Flatters and Garnett (309, Oxford Road, Manchester) have placed on the market a new immersion oil, known as mersol, which is entirely satisfactory, but I do not know whether this is prepared in the same way. Mr. Nelson, who mixed the materials for this oil so that achromatism and aplanatism were corrected, found it better than the immersion oil of Zeiss or Leitz.

Some time ago Rowntree (*Journal of Path. and Bact.*, Vol. 13, 1908), among others, suggested that liquid paraffin or parolein should be used in preference to cedar oil for immersion objectives, as it is cheaper, cleaner, perfectly neutral in reaction, and it does not dry up. He, however, admits that, on account of its lower refractive index, the microscope tube should be lengthened by about 7 mm. Other microscopists have been in the habit of using castor oil for the same purpose.

The question arises, Is liquid paraffin (R. Index 1·471), castor oil (R. Index 1·481) or thin cedar oil (R. Index 1·510) a satisfactory substitute for thickened immersion oil?

(" Note that the refractive index for the D line is, of course, important, but it is the dispersion value, or Greek ν value, that is equally important. When it is wrong, the tube-length will not correct."—*Nelson.*)

If you examine a slide, say, of *Spirochæta pallida*, either stained with Giemsa or prepared by Burri's method with Indian ink, with these substitutes as immersion oil, you will find that the image obtained with liquid paraffin or castor oil is an absolutely impossible one, notwithstanding the extension of the draw-tube to its extreme height.

As the result of a series of experiments I found, when liquid paraffin or castor oil was used as an immersion oil with the $\frac{1}{10}$-in. and $\frac{1}{12}$-in. objectives, that the tube-length had to be increased much beyond the range possible with any microscope. We may, therefore, conclude that neither liquid paraffin nor castor oil can possibly be used as an immersion oil, but that thin cedar oil is suitable, but not advisable. Incidentally, it may be noted that liquid paraffin has proved, as I first pointed out in 1911, one of the best mounting media for aniline-stained films. This was, apparently independently, confirmed by Giemsa in 1913.

The Oculars.

The most useful eyepieces, in my opinion, are as follows: The lowest ordinary or Hughenian, No. 0; it is better, has a larger field, and is considerably cheaper than the comp. oc. No. 2. It is especially suitable for searching.

" I find that Winkel's ordinary eyepiece No. 2 (which has power about 5) has much the same field as No. 1; this, of course, is caused by the size of the Continental tube. Therefore, I always use No. 2 for searching. I think that you will probably find the field of Leitz No. 1 eyepiece about the same as No. 0, in which case No. 1 would be the better for searching. I think you will find that No. 1 is the limit imposed by the Continental size of tube."—*Nelson.*

The others might well be compensating oculars, of which Nos. 6, 8, and 18 are the most serviceable. The compensating eyepieces, though primarily designed for use with the apochromatic, can be used with advantage on the higher achromatic lenses.

For photomicrographic work I have only used compensating oculars, and have had no experience with the projection oculars, which are considered by many to be more suited for this kind of work.

Mr. Nelson has given the following useful information on eyepieces in the *English Mechanic* :—

" With reference to the **multiplying power of an eyepiece,** this belongs to the A B C of elementary microscope management. It depends on tube-length—a five-power eyepiece with a ¼-in. objective on a 10-in. body will have a multiplying power of five; but if the eyepiece is used on a modern microscope with an intermediate length of body its multiplying power will be less, and if on the still shorter Continental body it will be less again. There is, however, another feature about this, viz., that, while the multiplying power of this eyepiece with a 10-in. body remains approximately five, with medium and high-power lenses, it will not be so with the lower powers, because the optical tube-length is not the same as the mechanical length of the body. Low powers throw the point, from which the optical tube is measured, up the body, so an eyepiece with a multiplying power of five with a ¼-in. objective may have one of only three with a 2-in. on the same body. These are elements known to most microscopists, and they form the reasons against engraving the multiplying powers on eyepieces.

" **The constant of any eyepiece can be found very easily.** Put on any objective—a $\frac{1}{2}$-in. is a good one for the purpose. Determine the combined magnifying power at 10 in. by a camera or other apparatus. Measure the diameter of the field by the stage micrometer. These two quantities multiplied together give the constant.

" When the constant has once been determined the **combined magnifying power** can be quickly ascertained by dividing this constant by the diameter of the field. Example :—Required the magnifying power of an eyepiece whose constant is 6·3, with a 4-10 objective, the diameter of the field being 0·024 in. ? Answer :—6·3 divided by 0·024 is 262, the power required. The same eyepiece with a 2-in. objective has a field of 0·15 in. What is the power ? Answer :—6·3 divided by 0·15 is 42, the power required. Nomenclature of Eyepieces.—In England they are called A, B, C, and D, but on the Continent and in America they are known by numbers, 1, 2, 3, 4, 5, and 6. Nos. 2 and 4 are those commonly used by medical students and biologists."

The Nosepiece.

There are two methods of changing from one objective to another, viz., by means of a nosepiece or by objective changers. A dust-proof triple nosepiece is, undoubtedly, one of the most important of all the accessories of the microscope, and absolutely indispensable for the real worker, but it must be well-made, and an object found with one objective must come fairly in the centre with another.

In some cases, if an object is found with the $\frac{1}{3}$-in. and then placed in the centre, it will not even be in the same field with the $\frac{1}{12}$-in. I know it is almost impossible to make a nosepiece which will exactly centre all three objectives, and, even if such were possible, it would only be absolutely correct for objectives by the same maker, as the thread of different lenses might vary slightly.

I have a Leitz triple nosepiece which will centre the $\frac{2}{3}$-in., $\frac{1}{3}$-in., $\frac{1}{10}$-in., $\frac{1}{12}$-in., $\frac{1}{16}$-in. objectives, all by the same maker, almost perfectly, and an object found with either will be in the field of vision, and fairly central even to that, when an 18 eyepiece is used.

Take the case of a bad nosepiece. A very minute object, the nature of which is unknown, is "spotted" during some investigation, the $\frac{1}{12}$-in. is turned on, and in all probability the unknown object will be quite out of the field, and it may be impossible to find it, because it will not be easily recognised with the increased magnification. This is not an imaginary case, but one seen every day.

I should be prepared to spend a considerable sum to have such a perfect nosepiece as I possess, and should certainly scrap one that was not reasonably good. I find it a good plan to mark on the nosepiece the figures 1, 2, 3 and try the three objectives on one of the six possible positions till the best arrangement is found and a note of it made. It should be noted that the centring of the $\frac{1}{3}$-in. and $\frac{1}{12}$-in. is by far the most important.

It may be asked, Why not have the objective changers of Zeiss and other makers, in which each objective is correctly centred before it is pushed home? All I can say is that they may be excellent for the *dilettanti*, but for the real worker I should regard them as perfect abominations. Who would be bothered to open a box, take out another objective, and place it on the microscope every time one saw something that might be of importance? It would be far better to have even an indifferent nosepiece, especially if one learnt in what direction the object usually went.

The Microscope Lamp.

It may be stated at once that no critical work can be done at the microscope with daylight, and further, as Mr. Nelson has pointed out, "One hour of steady hard work with the microscope by diffused daylight will tire you more than a whole day's work in a dark room by lamp-light."

For all forms of microscopic work, including dark-ground illumination and photomicrography, I have discarded all other forms of illuminants, and have come back to the paraffin lamp.

Any lamp with an $\frac{1}{2}$-in. or $\frac{3}{4}$-in. flat wick fitted with a chimney which has straight sides, such as what is known as a night-light chimney, will, as Ainslie has suggested, do; but it is better to have a good microscope lamp with ½-in. wick fitted with a black metal chimney with a 3-in. by 1-in. or 3-in. by 1½-in. glass slip. This was invented by Mr. Nelson, who points out the importance of not having the cistern too small; otherwise the oil may boil.

Such a lamp may be obtained from any of the microscope makers, but even then it will require some modifications. Almost all the lamps figured in the makers' catalogues have numerous fitments which are quite unnecessary, and which add considerably to the cost, but those which I should regard as essential are often absent.

A lamp need not be so elaborate, heavy, and expensive as some are. The essential points in a good lamp are:—

1. The flame can be turned with the edge or the flat surface towards the microscope without the observer even moving his eye from the eyepiece.

2. That any movement upwards of the lamp, which is very seldom necessary, should be accomplished without any elaborate rackwork.

3. That there be some method of attaching a bull's-eye so that the latter can be rotated in or out of the field, and which shall be in perfect optic axis when in position, without the observer taking his eye from the eyepiece.

4. That the chimney can be lifted up and rotated out of the way of the flame when not in use.

5. That the distance of the bull's-eye from the flame shall be constant. This, perhaps, may not meet with the requirements of all.

I find it can be placed either 2 in. from the flame—and this is the position I prefer for general purposes—or, as Mr. Nelson suggests for dark-ground illumination, as near the flame as possible.

The methods I have adopted to meet these requirements are shown in the photograph of the microscope and lamp on the microscope board. The upright support is placed on the left side. (Plate II.)

The oil reservoir, made locally, is circular, about 3½ in. in diameter and 2 in. deep. Quite in its centre the ordinary microscope lamp-burner, with ½-in wick, is fitted.

All the filigree brass clips, which are supposed to hold the chimney on, are cut off all round. A metal arm is made, fixed at one end to the middle third of the chimney and at the other end sliding on the upright, so that the chimney, when in position, rests by its own weight perfectly vertically over the burner, and it can be easily lifted up and rotated out of its position when not in use.

The metal chimney should always be turned aside when not in use, otherwise it may smell when the lamp is relighted.

I was anxious to increase the width of the flame of the ordinary microscope lamp, in order to get a slightly wider flame image for photomicrographic work, and, incidentally, to increase the amount and the area of light for dark-ground illumination.

Finding that none of the makers had such a burner, a friend kindly made the tube carrying the wick of my lamp just twice the previous width, so that now I use a 1-in. wick, folded longitudinally and lightly sewn, so that the wick is now ½ in., and double its former thickness. The image of the flame with this is distinctly wider.

It is advisable to use the best quality oil, and the addition of 2 to 3 per cent. of camphor is said to improve the actinic properties of the light.

Flickering or unsteadiness of the flame may be due to want of cleanliness, improper trimming of the wick, or imperfect oil or wick, and is usually easily remedied.

Sometimes the flame has a tendency to bend to one or other side; this usually means that there is a greater draught of air on the convex side, and can be remedied by drilling a small hole in the under surface of the chimney on the other side.

If the glass is constantly breaking, it may be that it fits in its grooves too tightly. Sometimes it is due to the bending of the edge of the flame forward, and in this case it can be cured by drilling two small holes in the front of the bottom of the chimney.

The Bull's-Eye Condenser.

There are several varieties of these on the market: The ordinary large single lens, the Nelson's aplanatic, and a very perfect but expensive one, the Watson-Conrady achromatised aplanat. I have the Nelson aplanatic condenser, but only use it for low powers. It is about 2½ in. in diameter and 1 in. thick. By far the best, and certainly the cheapest, bull's-eye condenser that I have ever used is the field-lens of an ordinary No. 2 eyepiece, which has, I believe, a focal length of about 2 in. Mr. Nelson suggested it to me, and the amount of light it passes is quite extraordinary. It is small and thin, and absorbs much less light than the large, thick condensers, and it can be easily fitted to the lamp.

The plane surface is placed towards the flame and, in order to determine at what distance from the lamp it should be fitted, I incline the microscope and project, by means of the mirror, the edge of the flame on to the ceiling, and move the lens to and fro till I get the sharpest image. (The bull's-eye condenser for dark-ground illumination can either be used at that distance, or, as Mr. Nelson has recently suggested, at a position as close to the flame as possible.) The bull's-eye is very generally regarded as rather a nuisance. Even Spitta says, "We confess at times to have found the bull's-eye more trouble than it was worth," and, when speaking of its use, remarks, "It had better be fidgeted about so that its position does not spoil definition, which it may do if incorrectly placed."

Mr. Nelson very truly says, "The bull's-eye should be fixed to the lamp, so that both may be moved together and not independently. To set up a bull's-eye on a separate stand correctly would take an experienced microscopist a quarter of an hour or more, an inexperienced one an evening."

The bull's-eye is an absolute necessity in my work and to most other workers, and it is nearly always in use for ordinary, and especially for dark-ground illumination.

The following seem to me to be essential in the fitment of the bull's-eye or accessory condenser :—

1. It must be fixed to the lamp, so that it rises and falls when the lamp is raised or lowered.

2. It must be placed at the correct distance from the flame and fixed there. For dark-ground illumination it can also be placed as near the flame as possible.

3. It must lie truly in the optic axis, at right angles to the rays of light coming from the lamp, whatever be the inclination of the microscope or the height of the mirror.

4. It must be capable of being placed in position, or removed out of position, without the observer taking his eye from the eyepiece. In short, it must be automatic, fool-proof.

After long consideration I have designed an attachment by which the bull's-eye can be rotated in or out of the field with the greatest ease, and when in position it is in absolutely correct alignment, whatever be the inclination of the microscope. My friend, Mr. Wm. Harding, kindly made this for me.

The small field-lens is fitted to the end of a brass rod, about 2 in. long, by a short arm which has a third of a circle of rotation, so that the lens can be placed in or out of the field. The brass rod has a hinge joint at the other end, at a point which corresponds exactly to the centre of the flame, so that it can be moved upwards or downwards, according to the level of the mirror. A reference to Plate II. will make this clearer than any description.

The method of using the bull's-eye condenser is described later.

Stops for Dark-ground Illumination.

In order to obtain all the slight variations in the size of the stop, so necessary for dark-ground illumination, I would strongly recommend the beautiful, but delicate, Traviss' expanding stop, which is now made by Watson.

It should be noted that the size of the Traviss' stop required depends on, first, the size of the condenser in use (the universal will require a considerably larger stop than the parachromatic, and I find the most suitable variation in size for the latter is about 6 to 14 mm.); secondly, on the size of the stop carrier, so that it may happen that a large stop carrier will require a stop with a range of small size, or *vice versa*.

When ordering an expanding stop, the size of the stop carrier and the nature or name of the sub-stage condenser should be given, or, if difficulty is experienced, the whole of the sub-stage apparatus should be sent to the makers.

Another point of importance is, that when the stop carrier rotates in from the right side the handle of the stop in the expanded position should be coincident with the handle of the stop carrier; when from the left side, the handle of the stop should be in a closed position at the same place. This, of course, is obvious when the apparatus is in position.

The ordinary three dark stops usually supplied with a sub-stage condenser are quite insufficient, as the very slightest difference in the size of the stop makes an enormous difference in its effects. By cutting out of thick black paper a number of discs of various sizes and sticking a suitable size, found by repeated trials, on one of the ordinary stops, I have made some fairly effective stops, but none are as convenient as the expanding stop.

Mr. Nelson suggested the use of a cone of stops, with very slight variations in size, which are fitted on to a starred disc, technically known as a spider, and Baker supplies these.

All the books state that the stop should be placed as close as possible to the back lens of the condenser. This is easily done in those forms of condensers which fit into the sleeve of the sub-stage from above, but in those which are pushed in from below, such as I have, there is some distance between the stop and the back of the condenser.

Personally, I have never experienced the slightest disadvantage from this, provided everything is perfectly central.

Colour Screens.

These are used to modify the illumination for visual and photographic work. They may be either coloured glass, or, better, specially-prepared coloured gelatine, or liquid colour screens.

All achromatic objectives are greatly improved by the use of a suitable green screen, and their performance then closely approximates to that of the apochromatic lenses.

Nelson, in the *Journal of the Quekett Mic. Club*, 1911, makes the following statement :—

" Formerly we all made a mistake by pitching our illumination too high up in the spectrum ; I am now speaking of visual, not of photographic, work. There can be no doubt about this, because fine detail is lost if the light is too high up in the spectrum. The cause is probably a physiological one. Experiments show that a normal eye is more sensitive in picking up fine detail when the light is a peacock green.

" Although with light high up in the spectrum the resolving power of a lens is increased, yet the sensibility of the eye is diminished. The art is to strike a happy mean between the two.

" The following are three lessons I have learnt :—

" 1. Not to work with light too high up in the spectrum.

" 2. Not to pass an opinion entirely on spectroscopic results.

" 3. Not to imagine that one screen is sufficient.

" Colours must be seen, but the following description of screens which prolonged experiments have proved to be the best, may be of service :—

" For daylight a piece of peacock green, worked down so that it is not too deep in tint, is combined with a very light-blue glass, not deeper in tint than a rather pale lilac petal.

" For lamp-light a thicker piece of peacock green plus a blue screen somewhat of the tint of a blue flower (*Centauria Cyanius*) common in cornfields.

" The ideal screen for visual microscopic work is one which, filtering out the too pronounced red, softens down, but does not entirely cut out, the orange and yellow lights.

" Twenty-five years ago any screen which did not pass certain spectroscopic tests by absolutely cutting out all light longer than a definite wavelength was rejected ; now we know better.

" The fact is that our heads were swelled by the table of resolving powers published on the cover of the *Royal Microscopic Journal,* where the three selected lights had wavelengths of 5,269 for visual line F, 4,861 for screen, and 4,000 for photography. I altered the visual to Gifford's maximum 5,607, and the photographic to 4,341 ; this last should be brought still lower down the spectrum to the photographic maximum through glass of 4,603, and the screen placed, at least, as low down as 5,184, if not lower."

Coloured glass screens are convenient, but not very satisfactory, for photomicrography, as they act as general absorbers, so there is always a considerable loss of light.

Wratten and Wainwright's mounted coloured gelatine are excellent, and by far the best for visual and photographic work.

The liquid screens are the most luminous, but are somewhat troublesome.

We have to remember that most of our specimens, especially those of bacteriology or protozoology, are purposely stained in several colours, and a deep colour screen takes away much, if not all, of the differential staining.

I use and recommend the following :—

1. A blue glass screen ; ordinary blue glass of the shops is not as good as a special light-blue glass which is obtained from Baker. This is the screen that I have constantly on the microscope, as it gives a soft daylight effect by which all stains are easily seen.

2. Wratten's green screen * B, used alone for visual and in conjunction with the yellow screen G for photomicrographic work.

As the B and G screens together require rather a long exposure, I now always use a lighter green-yellow screen, known as B3, No. 56.

The best position for a colour screen is in an additional stop-carrier fitted below the ordinary stop-carrier.

It can also be placed, in some sub-stage fittings which are pushed in from above, in the space occupied by the stop-carrier, but, of course, it has to be removed when the latter is in use.

A third position is by means of some mechanical contrivance on the tailpiece of the microscope. Lastly, it may simply be dropped into a slot in the light-screen which stands in front of the lamp.

Coloured gelatine films can be obtained either mounted between glass or unmounted. I have also mounted a green and a yellow B and G screen in Canada balsam between two glasses.

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* The best Wratten screen for observational purposes is that known as " Minus Red 4 " (Nelson).

The Wratten screens are to be obtained from Kodak, Ltd., who issue a useful little booklet on the question of screens or colour filters.

The Microscope Board.

Some years ago I fixed my microscope and lamp on a board so that everything should be in perfect alignment once and for all, and I have found this method so invaluable for general work, for photomicrographic work, and for camera lucida work that I would strongly advise others to adopt this or a similar device.

It saves an enormous amount of time and trouble, everything is in position, and almost every accessory to hand.

In order to start work all I have to do is to light the lamp, remove the Bell glass, and incline the microscope—a matter of a few seconds.

I venture to give some details of it, so that others may be induced to follow the same plan.

A piece of good rosewood or mahogany is chosen, about ⅝ to ¾ in. thick, 16½ in. long, 10 in. wide at the broad end, and about 6 in. at the narrow end.

A circular groove is cut at the broad end, 9¼ in. in diameter, $\frac{5}{16}$ in. in width and depth, to take the Bell glass.

In the circle grooves or slots are cut to admit the foot of the microscope, so that the instrument is firmly embedded in the wood. At the other end similar grooves are cut to receive the lamp and its upright stand.

The stand of the lamp is screwed or clamped into position, whilst, to prevent the microscope moving, a screw passes through from the under side of the board to a screw hole, which is found in the foot of this particular microscope.

Thus, the microscope and lamp are firmly fixed to the board, but what is equally of importance, should one wish to use the microscope directly without the mirror, the former is in direct line with the edge of the flame.

I determined this as follows: " After removing the eyepiece, objective condenser, and mirror, I screwed into the nosepiece the funnel stop used for dark-ground illumination with the

$\frac{1}{12}$-in., racked out the microscope to its highest, with the draw-tube fully extended, and placed on the top of the latter a tightly-fitting pill box pierced in the exact centre with a very small hole.

The microscope is then inclined, and the lamp moved about till the edge of the flame is seen in the centre of the very small field. The lamp is then fixed permanently in that position.

A curved piece of wood drilled with five holes, to receive four eyepieces and the object-marker in its case, is fitted on the left-hand side. In front, out of the way of the mirror, two larger holes are sunk in the wood to hold two additional condensers. On the right-hand side, near the lamp and outside the globe, is a hole for the oil immersion bottle.

Lastly, in front of the lamp is placed a piece of wood with a groove, to hold the shade which protects the eye from the light of the lamp. The distance from the centre of the flame to the mirror varies from 6¼ to 7¼ in., according to the inclination of the microscope. The under surface of the board is covered with velvet, so that the whole moves readily over a smooth and polished surface. If the microscope is to be used on a tablecloth the under surface should be left smooth.

The board does away with the need of a revolving table for the microscope, as it is easy to glide it along an ordinary table. The general appearance and relative position of microscope, lamp, and accessories are well shown in Plate II., for which I am indebted to my friend Mr. N. Jennings.

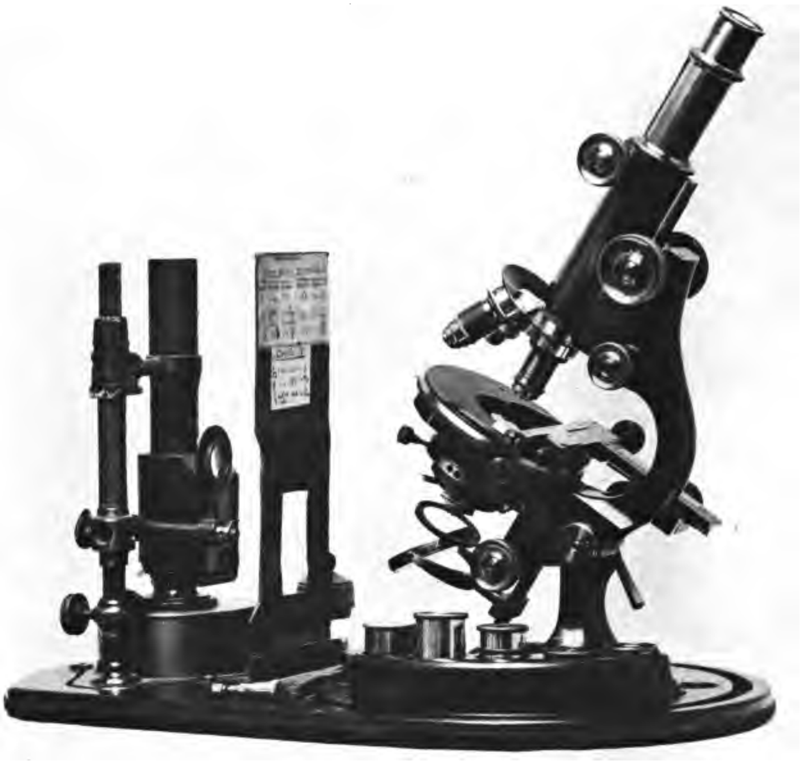
On the microscope board I have three accessories, which, though apparently unimportant, are actually indispensable.

1. **The object marker** is by no means a luxury, but an essential to one doing research work, as a means of marking the position of any special object for future reference. It was an English invention.

I use a Leitz instrument, which screws into the nosepiece like an objective and has a small diamond, which on rotation cuts a small ring on the cover-glass or on the film. It is fitted with a light spring, and a screw for adjusting the size of the ring.

Its use is as follows: I have found, say, with the ⅓-in. a spirochæte, I remove the ⅔-in. objective and screw in its place the marker, I then rack down till the diamond touches the slide,

PLATE II.



THE MICROSCOPE BOARD.

(*Facing p.* 30.)

give one circular turn of the marker, rack up again and continue work with the $\frac{1}{3}$-in.

Before the slide is moved the position is logged, vertically and horizontally. I adjust the side screw, so that the ring on the slide is well in the field of a $\frac{1}{3}$-in. and 6 comp. ocular.

Unfortunately, the rings on the cover-glass are not very easily found, except by racking down the condenser to diminish the light and focussing the surface of the cover, not the object.

The rings on the cover-glass cannot be found with the $\frac{1}{12}$-in., as the oil renders them invisible.

The plan I always adopt is to examine the slide without a cover-glass, either with the $\frac{1}{3}$-in. or $\frac{1}{12}$-in., and make the ring on the film itself. When the examination is finished, I remove the oil with xylol, and mount in the usual way in liquid paraffin. The rings are thus on the surface of the film, not on the cover-glass, and the very smallest object can be found when needed.

As the ring is on the film there is no need for the marker to be fitted with a diamond, and I should prefer one without it. I have never succeeded in making the rings on the cover more easily seen, but it might be possible to fill up the very fine rings with some pigment which would not be readily removed by cedar oil.

2. **A miniature micrometer,** opening 10 mm., reading in 20ths mm., can be obtained from George Adams, 77—78, High Holborn, W.C. This is very useful for measuring the thickness of cover-glasses and slides, costing, pre-war, 3*s.* 9*d.*

3. **A small lens** used for reading the verniers on the scale of the mechanical stage and for inspecting the surface of lenses is very convenient. A watchmaker's glass, of about 3½-in. focus, is also useful for the latter and for numerous other purposes.

Dirt or **particles of dust** on any of the optical parts of the microscope must be located and removed.

If the tube be racked well up and then, whilst the eye is in position, racked slowly down, particles of dirt or dust, generally speaking, come into view in the following order: on the eyepiece, slide, colour screen, mirror, bull's-eye, and lastly, on the glass of the lamp. Dust on the eyepiece can be detected in a dim light, with the iris diaphragm closed. To tell on which lens it is present, rotate the whole ocular and then clean the top or eye lens, replace, and turn it again. If dirt is still present, unscrew the eye lens and rotate that, and if the dust remains stationary it will probably be on the lower or field lens. Remove this and clean it. Replace the clean eyepiece and rack gently down, if dirt is still present proceed in this order: move the colour screen slightly, rub the surface of the mirror, move the lamp glass and, lastly, move the bull's-eye. On one or other of these, dirt or dust may be detected by a slight movement.

If dirt is still seen, it is probably either on the objective or the condenser. Look at the back lens of the former by holding an ocular inverted over the eyepiece in the tube of the microscope, and when this is focussed unscrew the objective about a quarter or a half turn, watching the effect of this movement. If the dust moves with the lens, it is certainly on the objective; if it remains stationary, it is probably on the condenser.

For cleaning eyepieces or objectives, nothing is much better than an old cambric handkerchief, which has been thoroughly washed with soda, well rinsed and not ironed, but simply dried. If chamois leather be used it should have been well beaten and shaken, or even carefully washed, and kept only for this purpose.

If I think an objective is dirty, I examine the surface of the front lens with a simple lens or, better, with a watchmaker's glass, pointing the objective to a good light. Dirt or dust can sometimes be detected by looking through the objective towards a diffuse light, with the front lens held close to the eye. Any dust or opacity will then be seen by slightly rotating the objective.

If dirt be seen, I first blow on the surface with a Politzer's bag, and then gently rub the surface with the perfectly clean cambric cloth. If oil or Canada balsam be present, clean it carefully off, with the corner of the cloth slightly moistened with xylol. On no account should spirit be applied to an oil immersion objective.

I then examine the surface of the back lens in the same way and, if necessary, clean it.

It is a good general rule never to separate the lenses of an objective, as dust or dirt are usually either on the outer surface of the front or back lens, and if there be any opacity on the internal lenses, that will be a matter for the maker.

Notwithstanding this rule, if I could not get at the outer surface of the back lens, owing to the nature of the mount, I should certainly be inclined to take the objective to pieces. The top or outer surface of the back lens has almost always some dirt or dust on it if it has been in use for a long time.

It may be blown away with the Politzer's bag, or one may roll up a little point of clean chamois leather and remove it in that way, but if you cannot get at it, then, to my mind, the only thing to do is to unscrew the lens.

Nelson, however, says : " To clean the back lens of an objective, a camel's hair brush is useful. Certainly, objective lenses should not be separated except by experts."

THE MANIPULATION OF THE MICROSCOPE AND ITS ACCESSORIES.

Critical Microscopy.

As that great microscopist Mr. Eliot-Merlin has stated: " There is only one kind of true critical microscopy, *i.e.*, that so named, originated and demonstrated by Edward Milles Nelson for many years past."

Critical microscopy is the beginning and the end of microscopy, and is the only method whereby one can get the highest possible efficiency out of the microscope; it is the attainment of the concert pitch of the instrument.

Nelson has defined it as follows :—

" **Critical Image.**—The image of an object is critical when it is obtained by means of an objective of fine quality which has been placed in correct adjustment for that object by its screw collar, or by the alteration of the tube-length, and when the illumination is critical.

" **Critical Illumination.**—An object is said to be illuminated critically when it is placed at the apex of a solid axial cone, the aperture of which is not less than three-quarters of the N.A. of the observing objective."

How to obtain Critical Illumination.

It is assumed that the microscope is fixed to a board with the lamp in front of it, as has been described previously, and that the edge of the flame is directed towards the microscope.

Incline the microscope to a comfortable angle. It is to my mind of extreme importance that the microscope should be placed in such a position that the head of the observer is in as natural and comfortable an attitude as possible. To work for any long period with the tube of the microscope vertical, whatever be the position of the observer, is extremely tiring. No wonder that so many workers get aching of the eyes or a headache when, as one so commonly sees in laboratories, they sit on a high-legged stool and lean over a vertically placed microscope.

As the front of the eyeball is constantly moist, when the head is inclined over a vertically placed microscope the fluid tends to run down over the surface of the cornea, and so impairs very considerably the vision, and in some cases even moistens the top surface of the eyepiece.

The vertical position of the instrument is exceedingly rarely necessary; a few very wet preparations may require it, but even then, if the cover-glass is closely applied, they can be examined in the inclined position.

It is not even necessary when using the camera lucida, as the surface of the drawing board has merely to be inclined parallel to that of the stage. Those who have to make many blood counts soon appreciate the fatigue resulting from using the microscope in the upright position, but I find that, when the corpuscles have sunk to the bottom of the counting chamber, the instrument may be slightly inclined to a more comfortable position, provided the movement is made carefully and without any jerk.

One would be surprised at the angle to which the counting chamber can be inclined until it has been tried.

The position of the observer should be one of the maximum degree of comfort. As a matter of fact, I do all my prolonged work seated in an armchair. I have had a special stand made, which is simply a piece of stout gas tube, 1⅜ in. in diameter, screwed into a heavy cast-iron foot, part of a reading stand, which has a spread of 18 in. At the top of the upright a circular mahogany disc, 12 in. in diameter and 1 in. thick, is fixed at a height of about 23 in. from the floor.

Recently I have had the top part made to telescope in or out a few inches, and this is a great convenience. The table fits closely up to the armchair, and the microscope and lamp, which are fixed on to a board, rest very firmly on its surface.

The mirror, when the microscope is inclined, should be on about the same level as the flame of the lamp. If the latter be not fixed, it may be raised or lowered, or, as I prefer, the lamp remains stationary and the mirror is pushed up or down the tailpiece. In my opinion the exact level of the lamp and mirror is not of much importance, provided the mirror stem is not too long, a defect very commonly present, as I have previously described.

1. Place on the nosepiece a ⅔-in., or, better, a ⅓-in. objective, and use a low eyepiece. Place any object on the stage.

Fill the field with any kind of light by tilting the mirror, and focus the object and bring it into the centre of the field.

2. **Centre the Condenser.**—This can be done in two or three ways. (*a*) Keeping the eye on the eyepiece, close the iris diaphragm, and rack down the condenser till the hole formed by the margins of the iris is clearly seen; then, by means of the centring screws of the sub-stage, bring the image of this hole central with the field of vision. It is advisable, especially when centring the $\frac{1}{12}$-in. by this means, to remove the colour screen, if one be present, in order to get more light.

It sometimes happens that it is impossible to see the opening in the iris even with a low power; this depends on the focus of the condenser.

Watson's holoscopic condenser, for example, is a ⅓-in. objective, consequently the iris can be focussed because it is relatively far off. If the condenser were ½-in., with the iris close to the back lens, it would not be focussed unless it were placed 2 or 3 in. lower down, and this cannot be done on any continental stand. (Nelson.)

When it only occurs when the $\frac{1}{12}$-in. is used, it is probably due to the fact that the iris does not close up to a sufficiently small size, and the remedy is to use the lowest eyepiece and the shortest tube-length in order to get the lowest magnification possible.

If this fails, then centre the ⅓-in., as described, and centre the $\frac{1}{12}$-in. from this.

(*b*) The converse of the above. Focus the image of the edge of the flame on the object; then close the iris, and rack up the objective with the coarse adjustment till the small opening in the iris diaphragm is seen. If the objective and the condenser are in true alignment, the result will be precisely the same as in the first case (*a*).

I almost invariably use the first method (*a*), except when an oil immersion condenser is oiled to the slide. Racking down the condenser would break the oil contact.

If the $\frac{1}{12}$-in. objective is being centred on a slide which is in oil contact with the condenser, the objective should be racked down till it almost touches the slide, no oil being used, and the iris closed to its smallest, and then the objective is slowly racked up till the opening in the iris is seen. Even this method is not so good for this purpose as the next.

(*c*) Having focussed the object and the image of the lamp flame, examine the back lens of the objective, either directly by removing the eyepiece and looking down the tube, or, better, by retaining the eyepiece in position, examine the Ramsden disc by another eyepiece held inverted a short distance from the ocular. If at the same time the iris be slowly opened or closed, its margins can be seen and the iris opening centred by the centring screws of the sub-stage. Assuming the microscope is fixed to a board, and the lamp is similarly fixed in front in the true optic axis, how could you roughly tell, by looking down the microscope with any eyepiece and any objective, whether the condenser was central ?

(i.) Under these conditions, if the condenser be central, the image of the flame, when placed in the centre of the field, lies about north and south, or from twelve o'clock to six o'clock.

If the condenser be not central, when the image of the edge of the flame is in the centre of the field it will be slightly oblique, say, from eleven to five o'clock, or from one to seven o'clock.

(ii.) On opening or closing the diaphragm it will be noticed that if the condenser be central the shadows move in equally on both sides of the image of the flame ; if the condenser be not central, they move in unequally.

Of course these tests show only lateral, but not much antero-posterior, displacement of the condenser.

3. Focus the source of light by racking up the condenser until the image of the edge of the flame comes into focus with the object, and the flame image should then be placed in the centre of the field by slight movement of the mirror.

If the image of the flame is unsteady, flickers, or shows several reflexions, correct this as described earlier under the mirror.

4. If it is desired to use a higher objective, turn on, say, the 1/12-in. and focus the object again. If the object and the image of the edge of the lamp flame are not central to the new field, first place the object in the centre of the new field, and, by means of the centring screws of the sub-stage, bring the flame central to it. On no account must the mirror be moved for this purpose.

Of course, if the alignment of the nosepiece were perfect, it would not be necessary to recentre the object and the image of the flame, but, unfortunately, it is seldom as perfect as that.

This is one of the methods of centring the 1/12-in. after the condenser has been centred for a lower power, say, the ⅓-in. objective.

To my mind this method has the disadvantage that, whilst it is easy to detect lateral displacements of the edge of the flame, it is difficult to see any deviation of the flame upwards or downwards. Personally, I prefer to use the first method (*a*), even for the highest powers.

5. What **cone of light** shall we use for an objective whatever be its power or numerical aperture? Nelson has definitely established, and other microscopists have at last agreed, that, whatever be the power of the objective, only three-quarters of its aperture should be used. The better the objective the greater the size of the cone that can be utilised.

In a letter to me some years ago he says: "There are very few lenses that will stand an illuminating cone of more than three-quarters of their full aperture. Now, your 1/12-in. is N.A. 1·3, and three-quarters of this is 0·975; therefore, a dry condenser N.A. 1·0 is sufficient for your purpose. In other words, if you can use a cone of N.A. 1·0, you are utilising more than three-quarters of your aperture, and yours must be a superlatively fine lens to stand it.

"First-rate apochromatics will stand more than semi-apochromatics, and so will semi-apochromatics than achromatics. I have seen a very few apochromatics that will stand a ⅚ cone.

"Of course, you get out of a lens what you put into it. The question of importance, when recording an observation, is not what the lens is with which the observation was made, but what was put into it. For example, a statement that a certain object was seen with a Zeiss apo. $\frac{1}{12}$-in. N.A. 1·4 by itself means nothing. Perhaps the lens was worked with an aperture of 0·3, and so the lens with an aperture of 1·4 was degraded to an aperture of a dry lens, say, an ordinary $\frac{1}{2}$-in. or $\frac{1}{4}$-in., but an observation that such a lens was used with a cone of 1·0 or of 1·1 means a totally different thing. Then, if the lens is a good one, it will stand it, and the highest possible resolution will be obtained; or if, on the other hand, it is a bad one, it will break down, and nothing will be seen."

These facts are interesting when you see so many men buying an apo. 2 mm. of N.A. 1·4 and using it, not with an achromatic condenser of about N.A. 1·0, but with a common Abbe chromatic condenser, which only gives an aplanatic cone of about 0·5 !

If " the effective N.A. of the objective is a mean between its own N.A. and that of the illuminating cone, at any rate as far as its resolving power is concerned " (Ainslie), then an apochromatic lens of 1·4 N.A. used with an ordinary Abbe condenser of 0·5 aplanatic N.A. $= \frac{1 \cdot 4 + 0 \cdot 5}{2} = 0 \cdot 95$ of effective N.A., whilst with a $\frac{1}{12}$-in. of 1·3 N.A. (costing one-fourth of the apo.) used with an achromatic condenser of, say, N.A. 0·95, the effective N.A. would be 1·12.

How to Ascertain the Size of the Cone.

With the object and the edge of the flame in focus and the condenser centred, examine the back lens of the objective.

This may be done in two ways :—

1. Remove the eyepiece and look down the tube of the microscope, when any movement of the iris diaphragm can be seen. This examination can be made somewhat easier by means of a dummy or blank eyepiece, which is simply an ordinary eyepiece mount having no lenses in it, with a small aperture in the cap. It can be easily made by getting a pill-box which just fits the top of the tube of the microscope and boring a small hole with a thick needle in the centre.

It is quite easy to see the back lens by either of these means when a medium or low power lens is being used, but in my experience somewhat difficult when a $\frac{1}{12}$-in. objective is in position, and I prefer the following method :—

2. Place the lowest eyepiece, say, a No. 0 or 1, in position. Hold over it any ocular (except comp. oc. 8 or 18) inverted at a little distance, and focus this by placing it nearer or farther away till the margins of the iris are distinctly seen. By this means a magnified image of the Ramsden disc is seen.

I find with Leitz $\frac{1}{10}$-in., $\frac{1}{12}$-in. and $\frac{1}{16}$-in. oil immersion lenses that the comp. oc. 8, which is a positive lens, when standing erect on the top of the ordinary eyepiece No. 0, gives an excellent view of the back lens, especially when it is focussed by varying the tube-length of the microscope. (I learn that, unfortunately, they are now making the comp. oc. 8 a negative one, and without a cap.)

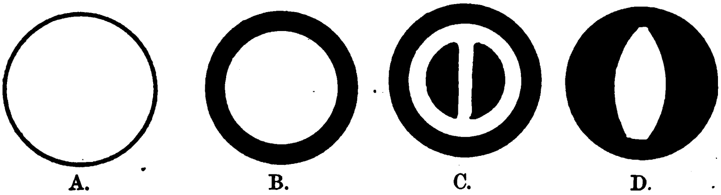


DIAGRAM OF THE BACK LENS OF AN OBJECTIVE AS SEEN WHEN THE EYEPIECE IS REMOVED, OR BY AN INSPECTION WITH A MAGNIFYING LENS OF THE RAMSDEN DISC.

A. Full cone. B. Three-quarter cone. C. and D. When the sub-stage condenser is racked up within its focus, or when a bull's-eye has been placed between the light and the sub-stage condenser and not properly focussed.

To obtain the correct cone, close the iris till three-quarters of the back lens is full of light (Fig. B). This rule is perfectly correct for any dry lens, but it is not quite correct for an oil immersion objective, unless an oil immersion condenser be used.

When you are using a dry condenser, the very highest N.A. you can get is 1·0, whilst the objective has an N.A. of 1·3 ; on the other hand, with an oil immersion condenser, the N.A. may be 1·3, and then the above statement would be right. The rule, therefore, is, when using an oil immersion objective with a dry condenser, close or open the iris diaphragms till the margins are just visible at the margin of the back lens (Fig. A).

Although it is a cardinal rule to use three-quarters of the aperture of a good objective, it may be found necessary, when examining transparent and unstained objects, to somewhat close the iris.

After a time, with practice, one can feel by the position of the handle of the iris diaphragm approximately the spot at which a three-quarter cone is obtained for the $\frac{1}{12}$-in. and the $\frac{1}{3}$-in. objectives.

So far we have centred the condenser, have focussed the object and the flame, and have seen that the iris diaphragm has been closed to a cone of three-quarters of the objective.

Are we not now in a position to get the very most out of our object-glass? No, certainly not. We have yet to take into consideration the thickness of the cover-glass and, to a less extent, the refractive index of the medium in which the preparation is mounted, one of the most important of all the adjustments.

Tube-length Adjustment for Thickness of the Cover-glass.

The correction of an objective either by means of an alteration in the tube-length or by means of the correction-collar is, in my opinion, of the very greatest importance in microscopy.

It is a subject which has received very inadequate treatment by the text-books, and one which has been completely ignored by very many microscopists.

Mr. Nelson, writing to me some years ago on the question of tube-length, says: "When an eye becomes trained, a lens out of correction offends it just as much as one out of focus would the general worker.

"If an object were shown to a biologist out of focus, he would directly put it in focus by the fine adjustment; in the same way an over-corrected or under-corrected image is perceived by the trained eye, and must be corrected by tube-length alteration. Mr. Powell had, twenty-five years ago, a most wonderfully keen eye, and 1 mm. of alteration of the tube-length would be detected by him.

"Tube-length is only another name for lens adjustment.

Lens adjustment is the keystone of microscopical work, and it requires many years of constant practice to acquire a knowledge of it. Low powers with the same N.A. are more sensitive than high powers. This is shown by my term O.I.—optical index. The greater this value, the more sensitive the lens.

" Lens adjustment is a more important factor in microscopical work than any adjustment of the condenser or of the lamp distance, etc.

" An apochromatic condenser with collar adjustment to correct for thickness of the slide, and the most refined lamp, etc., will not be of any use unless the objective adjustment is correct."

Mr. Nelson has kindly sent me the following contribution on this important subject :—

Lens Correction.

" A microscope objective is designed so that it shall be in correct adjustment when the object is covered by a cover-glass of a certain thickness and of a certain refractive index, or rather, which is more important, a certain value of $\delta\mu$. If, therefore, when any object is being examined, either of these quantities differ from those for which the object glass has been designed, the object glass will be out of adjustment, and the image of the object will be imperfectly presented to the eye of the observer. Now there are two methods by which the lens can be approximately readjusted for objects covered by glasses, differing from that for which the object glass was designed.

" The first, and best, is by means of a rotating collar on the objective itself. Rotation of this collar causes the back lens of the objective to approach or recede from the front lens.

" The movement required is minute, so the mechanism, to be efficient, is delicate and must be very well made, consequently it is expensive ; formerly, all first-class objectives, from the inch upwards, were so fitted.

" The second method is by varying the length of the draw-tube, and in order that this might be accurately and easily performed, I had a rack work fitted to the draw-tube of my microscope forty years ago, and it is the first microscope that was so fitted.

The Meaning of Tube-length Adjustment.

"There is neither importance nor meaning in tube-length adjustment, *per se*, it is only a means to an end, and that end is working distance. Obviously, working distance means the distance of the front lens of the objective to the object, but it does not mean the focus of the objective.

"Every observer knows that when he makes his tube longer he has to refocus his objective by moving it nearer the object, and that when he shortens it he will have to refocus his objective up.

"Now it is this alteration of working distance that effects the needed correction for different thickness of cover, refractive index, etc. The alteration of tube-length is only the means by which this proper amount of working distance is obtained.

"To restate this important condition: The object glass has been designed and constructed to work at a definite distance, with a cover-glass of given thickness and refractive index. If a cover of different thickness, refractive index, etc., is used, then the object glass will require a different working distance, and to obtain that different working distance, either the back lenses of the objective must be moved, or if those remain fixed, the length of the tube must be altered.

"Correction by alteration of tube-length is much less costly than that by movement of the back lenses; it has, however, the disadvantage of causing great alteration in the power of the microscope, while the movement of the back lenses does this to a much less extent. To be precise, alteration of tube-length alters the focus of the entire microscope; alteration of lens position by collar adjustment alters the focal length of the object glass, as well as its working distance.

"A difference of 10 or 12 in. of tube-length is by no means unknown, and so great an alteration as that means a very great alteration in power. Medical men deal with objects for the most part mounted in more or less similar media, under cover-glasses more or less uniform in thickness, therefore the range of tube-length correction is not often excessive. It is most important that the condition, as stated above, should be thoroughly understood and remembered by the student, for it will save him from falling into error in his practical work.

"An example will illustrate this. An observer, provided that he knows his work, when he has once adjusted his objective, by altering the tube-length, will be very careful to maintain his working distance a constant quantity. Thus, if he changes his eyepiece, and thereby the image is made indistinct, he will not, as so many do, spin round his fine adjustment and refocus his microscope, because he knows that that would alter his working distance, and consequently put his objective out of adjustment, which he had previously taken such pains to adjust. But he would refocus by means of tube-length alteration, because that would leave his working distance at the same point as it was before.

"This tube-length focussing applies, of course, to the rough focussing, the minute focussing being performed by the fine adjustment."

If you ask many, I would almost say most, workers with the microscope what was the use of the draw-tube of a microscope, they would tell you that it was merely a means of getting variations in the magnifying power of an objective!

Give me an ordinary $\frac{1}{6}$-in. of average quality, and a superb $\frac{1}{6}$-in. of fine quality, and I will show you an image by the former at the correct tube-length incomparably superior to that of the latter when placed at an incorrect tube-length, and yet every day men are passing judgment on lenses examined under uncritical conditions of illumination, with the most imperfect condensers, and with a total disregard of the tube-length.

Every objective is corrected by the makers to work at a certain tube-length for a given thickness of cover-glass.

The usual standard thickness for cover-glasses is about 0·17 mm. Leitz uses 0·16 to 0·18 mm., Zeiss between 0·15 and 0·2 mm., and the thickness of the cover-glass for which the objective is corrected is marked in very small figures on the mount.

The tube-length for which objectives are corrected is for those of Zeiss and most English makers 160 mm., and for those of Leitz 170 mm. The figures on the draw-tube do not include the thickness of the nosepiece, which varies from 15 to 20 mm.

Spherical aberration in the form of under-correction is introduced in the objective when the cover-glass is too thin, and is remedied by lengthening the draw-tube, or if there be a correction collar, by opening the lenses, whilst, on the other hand, if the cover-glass be too thick there is over-correction, and this can be removed by shortening the tube-length or by closing the lenses of the objective by the collar.

The question may well be asked, how are we to know when the tube-length is correct for a given cover-glass ?

No doubt a very trained eye will soon see when the finest resolution is obtained by lengthening or shortening the tube, but there is another and an easier way than this by which one can determine more or less exactly the length of tube which will give the best image, and that is by observing the way in which an object comes in and out of focus. This, I understand, is a method known to, and used by, opticians for many years.

I have been in the habit for some considerable time of adjusting the tube-length for various thicknesses of the cover-glass, and to a less extent the refractive index of the mounting medium, by watching how a minute speck or a fine line appears when the objective is slightly, and in an equal degree, focussed up and downwards.

Commander M. A. Ainslie, R.N., F.R.A.S., on "Tube-length Adjustment" in the *English Mechanic*, June 18th, July 2nd and 9th, 1915, and also in a lecture on "Some Principles of Microscopy" in the *Photomicrographic Journal*, p. 10, 1916, has given such an excellent and detailed account of this method that I have taken the liberty of quoting here rather fully from these.

"Taking first the consideration of a point of light, such as is always found by dark-ground illumination, let us assume that the cover-glass is too thin or the tube-length too short. Use a fairly high dry lens, such as a $\frac{1}{6}$-in. of N.A. 80, and a fairly large central solid cone of illumination.

"Having focussed the image as well as possible, let it be thrown slightly out of focus by means of the fine adjustment, first in a downward direction, then in the reverse. If, as has been assumed, the tube-length is too small for the cover-glass employed, it will be found that when the tube is depressed the image of the point of light expands into a ring, the centre remaining dark, or, at any rate, showing a well-marked deficiency of light as compared with the margin. On withdrawing the objective from the object by raising the tube, the image takes on a characteristic and easily remembered ' soft ' or ' misty ' appearance, the centre remaining comparatively bright, while the margin becomes foggy and ill-defined, presenting a great contrast to the hard margin seen when the tube is depressed. This foggy appearance is so distinctive that I am in the habit of looking for it whenever I have to adjust the tube-length ; and once seen, it will not be readily forgotten.

" If, on the other hand, the tube is too long or the cover too thick, or both, then the appearances of the out-of-focus image, according as the tube is raised or lowered, are reversed ; the foggy appearance is seen on depressing the objective, *i.e.*, within the focus, and the ' hard ' appearance on withdrawing the objective, so that the focal plane of the eyepiece cuts the cone of rays beyond the focus of the objective. The appearances are just the same as in the case of the thin cover or the too short tube, only they have changed places.

" We have thus a means of telling whether the tube is too long or too short ; if the misty appearance of the out-of-focus image occurs when the tube is depressed, the tube is too long, and should be shortened ; if the mist comes on when the tube is raised, then the tube requires lengthening.

" Further, it is possible to give a very simple and easily remembered rule for the correct procedure ; having obtained the ' misty ' appearance, focus the image again, and continue turning the fine adjustment until the focus is passed and the ' hard ' appearance takes the place of the ' misty ' ; then, without touching the fine adjustment, restore the focus by moving the draw-tube. It will now be found that the image is distinctly improved ; it can be again tested by observing the out-of-focus discs in the manner already indicated, and at length it will be found that the appearance of the image, out of focus, is the same whether the tube is depressed or raised ; it is then clear that the rays of light from the objective all come to the focus at the same point, and that the tube-length adjustment is, in fact, correct.

" The image should, in the first instance, only be put slightly out of focus, just sufficiently to show the effects indicated. If it is put too much out of focus, re-focussing by means of the draw-tube may introduce the opposite error to that which it is desired to correct. As the adjustment approaches perfection, the ' misty ' effect is less noticeable ; but it is still present until the tube-length is correct, and its recognition is purely a matter of eye-training.

" The reason why I have recommended that the ' misty ' appearance should be looked for is that the ' hard ' is very much the same in character whether the tube-length is correct or not. When the tube is of the correct length, the ' hard ' appearance is seen on both sides of the focus, though to a much smaller degree, so it is as well to take as the sign of incorrect adjustment the ' misty ' appearance, which is, or should be, only seen when the tube-length is incorrect, and to work towards the elimination thereof.

" Up to this point we have been considering only the question of a single bright point of light, and as such are always to be found whenever dark-ground illumination is in use, it is a good plan to commence practising with this form of illumination ; but it is now necessary to consider the case of dark objects on a bright field as being that most frequently met with.

" To start with, let us consider the case of a single very minute black point.

" Such a point may be described as the ' negative ' of a small bright point, and it will probably be expected that the appearances can be deduced from those of a bright point by merely interchanging the words ' bright ' and ' dark.' This is the case, and if the cone of rays referred to above be considered a ' cone of darkness ' (or ' caliginiferous beam,' to use the not very elegant phrase of a well-known writer), it will be easily seen that instead of the ' misty ' appearance above described we shall have a darkish centre with a grey fog surrounding it, and in place of the bright margin and dark centre we shall have a dark, fuzzy ring, with perhaps a not very evident grey fog inside it.

"The rule for adjusting the tube is just the same; obtain the 'misty' appearance, pass through the focus to the other side, and then re-focus by means of the draw-tube. It will often be found that there are specks of dust on the slide, which, though not beautiful in themselves, are of great assistance in making the adjustment under consideration.

"The first attempts to put in practice this or any other method of adjusting the tube-length should be made with as nearly as possible the full aperture of the objective, and with an objective of large aperture; and the lower the power of the objective is, the more readily will the effects to be looked for be found. The best objectives to employ in learning to use this method are, undoubtedly, the ½-in. 'Holos' or the Zeiss ½-in. apochromat; but the former is perhaps, and the latter certainly, rather in the nature of an expensive luxury to most people, and an ordinary 1-6, of N.A. not less than 0·75, will be found quite sufficient, if it is borne in mind that with such the alteration of tube-length for any given case is much greater than in the case of the ½-in. Whatever the lens employed, it should at first, until the eye has begun to recognise readily what is to be looked for, be employed nearly, if not quite, at its full aperture; and an Abbe test-plate is the best object to practise on, or, failing such, a slide that shows plenty of dust, or other opaque spots. A well-stained slide of fairly large bacteria is also excellent. It should not be forgotten that it is necessary to practise with objects on a dark ground as well as those on a bright—indeed, more necessary, as in dark-ground work the objective is used at its full aperture, and the necessity for accurate adjustment of the tube is all-important.

"It may be mentioned, as arising from this subject, that a very fair judgment of the value of an objective may be got by observing whether the best tube-length is the same, whatever be the aperture of the sub-stage iris diaphragm employed. With a first-class objective, the correct tube-length found by this method ought to be the same whether the whole, or nearly the whole, aperture of the objective is in use, or whether, on the other hand, it is stopped down to one-half or two-thirds of the full aperture. With an inferior objective, on the other hand, it will often be found that if the tube-length has been adjusted, say, for a ' three-quarter ' cone, it will be necessary to alter the position of the draw-tube by several millimetres on closing or opening the iris."

To demonstrate the effects of variations in the thickness of the cover-glass, and to learn to correct the defects arising therefrom by means of alteration in the tube-length, I should suggest proceeding as follows :—

First, see that the draw-tube works smoothly and easily. Undo the milled-head screw just above the coarse adjustment, remove the draw-tube and thoroughly clean and polish it, and also the ring through which it works, if this is not lined with cloth. Next, see that the coarse adjustment works somewhat stiffly. In the Leitz microscope there are two steel screws placed over the axis of the coarse adjustment for this purpose.

The draw-tube should work as smoothly as silk, for, if it be tight and the coarse adjustment loose, it is quite easy to smash the objective and the slide when the tube is pushed in.

For test objects I should advise a slide of the *Spirochæta pallida* stained with Giemsa, mounted and one unmounted; and a mounted slide of the same organism prepared by Burri's method with Indian ink. If these are not obtainable or to hand, one can easily make a suitable test preparation from the spirochætes of the mouth.

With a toothpick or a pointed piece of match, pick a tooth, especially a decayed one, or scrape the gum where it joins the teeth, or the spaces between adjacent teeth of one's own mouth. Spread a very thin film of the material so obtained on two or three slides. When dry, stain them (as described at the end of the book) with Giemsa or Leishman's stain for, say, an hour. Wash, dry, and mount one in Canada balsam or liquid paraffin; keep the other film unmounted.

To prepare a preparation according to Burri's method, procure from the stationer's a bottle of Gunther and Wagner's Indian ink, that brand known as " Pelican " or " Chin Chin " ; no other Indian ink will do. Place a very small drop of this ink on one end of a slide and add about an equal quantity of the tooth-scraping; with the toothpick or a needle mix thoroughly, and with the edge of another slide spread out the film in as thin a layer as possible; when dry, mount in Canada balsam.

(*a*) Place the dry, stained, unmounted film containing spirochætes on the stage and examine it by one of the methods of dark-ground illumination described later, using for this purpose the apo. 8 mm. or ⅓-in. objective of N.A. 0·65, with either comp. oc. 8 or 18, or even an ordinary No. 4 or 5 eyepiece.

Focus carefully any small brilliant spot, then with the fine adjustment focus slightly upwards and equally downwards, noticing the effect. If a halo or ring appears round the object on focussing downwards, but not on focussing upwards, lengthen the tube, and go on increasing the length of the tube till equal-sized rings appear on focussing either above or below the correct focus.

Note the mark on the tube. Say that is 210 mm. Repeat the observation with various eyepieces till you have obtained about the same result each time. Then the tube-mark 192 + the thickness of the nosepiece, say, 18 mm., = 210 mm., the tube-length; and you may take it, as a rule, that all uncovered objects require for dark-ground illumination a tube equal to that length—in this case 210 mm., and in future cases you simply place the tube at that position without repeating the trials.

If your objective is a good one, you will also find that it will give the best result at that tube-length for an uncovered object, even for ordinary illumination, when you are only using three-quarters of the cone.

"Dark-ground illumination tests the full aperture of an objective, whilst ordinary illumination only tests three-quarters of the aperture, so that the correct tube-length for dark-ground illumination or full cone may not be quite the same as that for ordinary illumination with a three-quarter cone, and a different tube-length may be required by achromatic and semi-apochromatic, and, to a much smaller and perhaps unnoticeable extent, with apochromatic objectives."—*Nelson.*

(*b*) Remove the slide, drop on the surface a few drops of liquid paraffin, and stand the slide on its end for a few minutes, in order to allow the excess of the thin oil to drain away. Then replace the slide on the stage, if possible, in the same position and examine by dark-ground illumination.

You will notice that the object is not nearly as brilliant or as striking as in the dry condition, but you should have no difficulty in recognising that rings are present when focussing downwards, but absent on focussing upwards, if the tube-length be too short; whilst the rings are seen on focussing upwards if the tube be too long.

Adjust the tube-length till the rings are equal in both directions and you will probably find (at least, that has been my experience with two Zeiss and one Leitz apo. 8 mm.) that the position at which the rings are equal is the same as before.

In other words, a very thin layer of liquid paraffin, or, as a matter of fact, water, does not necessitate any alteration in the length of the tube.

(*c*) Remove the slide, place it in a bottle of xylol to remove the liquid paraffin, and, when dry, pour on a drop or two of immersion cedar oil. Let the slide drain for the same time as before.

Replace and examine it under the same conditions as before, and you will probably find that the tube requires to be a little shorter, owing chiefly to the fact that the more viscid oil forms a decided layer on the surface of the film.

Having found the correct tube-length for an uncovered object, examine the same object by dark-ground illumination and by transmitted illumination, with the tube an inch or two too short, and you will see, especially if comp. oc. 18 be used, that the image of the spirochæte is blurred, hazy, and " rotten," giving one the same impression as if you had breathed on the eyepiece. When, however, you return to the correct tube-length, the image of the organisms is crisp, sharp, and clear.

(*d*) Remove the slide again, place on the surface a drop of cedar oil, and apply a cover-glass, but do not press it down; let it lie lightly on the film. Examine, and you will find that the tube has to be considerably shortened before the rings are equal, both by transmitted or by dark-ground illumination. Say the rings are equal at the tube-mark 140. Remove the slide and press the cover-glass very firmly down, and remove all excess of cedar oil by blotting paper. Re-examine, and you will notice that the tube has to be lengthened, say, to a tube-mark of 150, before the rings are equal on focussing the object above or below the correct focus.

(*e*) Place the slide of *Spirochæta pallida* mounted in Canada balsam on the stage, examine it by dark-ground and ordinary illumination, and note the tube-length at which the rings are equal on focussing upwards and downwards. Then remove the slide, warm it gently over the flame of a spirit lamp, and try and squeeze out a little of the balsam. Re-examine, and it will be found that the tube has to be lengthened, because the thinner layer of balsam has the same effect as a thinner cover-glass.

(*f*) Examine the slide of spirochætes in Indian ink, at first with a dry lens and afterwards with an oil immersion objective, with critical illumination. The bacteria and spirochætes here appear white on a dark background, and the effects of an incorrect tube-length are almost as easily seen as they are in the case of dark-ground illumination, and this sort of slide is one of the best and most easily obtained for learning the method of arriving at the correct tube-length. The rule to remember is: rings downwards, lengthen the tube; rings upwards, shorten the tube.

Almost all the books state that the oil immersion objectives are but slightly affected by variations in the thickness of the cover-glass, working, as they do, through a layer of oil of about the same refractive index as the crown-glass of the cover-slip, and hence known as homogeneous immersion lenses.

My experience, rightly or wrongly, is that these lenses require some alteration of the tube-length, both for different thickness of the cover-glass and for different refractive index of the medium in which the object is mounted, before the best results are obtained.

When speaking of immersion cedar oil, I have drawn attention to the marked alteration of tube-length required for other oils, *e.g.*, liquid paraffin and castor oil, when these are improperly used as a substitute for the ordinary thickened cedar oil.

Ainslie states that " The correct tube-length (for immersion lenses) is of great importance, though independent of the cover-thickness, and it is almost always stated, once for all, by the makers of the objective. It is, at the same time, as well to check the figures given. I have recently examined a number of oil immersions, corrected for the 160 mm. tube; but, on trial, the correct tube-length was found to vary from 145 to 195 mm., there being considerable variations even in different lenses by the same maker.

"With oil immersions of high power, such as $\frac{1}{16}$ and higher, a small alteration (10 mm. or so) of tube-length does not make a very great difference to the definition; but with those of moderate power, such as $\frac{1}{8}$ and $\frac{1}{10}$, the correct tube-length is of great importance, and should always be carefully tested; once found, it need not afterwards be varied, even for great variations in cover-thickness."

I have purposely omitted to describe the method of using the correction collar, as the dry objective I so strongly advise, viz., apo. 8 mm. or $\frac{1}{3}$-in., is not fitted with one.

The apo. 4 mm. or $\frac{1}{6}$-in. has a correction collar, but I have pointed out elsewhere that this lens, at least in its existing condition, is quite useless for the examination of uncovered objects, and its high N.A. of 0·95 also prevents its use with dark-ground illumination obtained by means of a dry condenser with a stop.

The Method of Using the Bull's-eye Condenser.

So far we have obtained a critical image with critical illumination, by means of which we obtain the highest possible resolving power of the microscope. But, you may say, the area of the field illuminated by the edge of the flame is so small, especially when the $\frac{1}{3}$-in. objective is used with a low eyepiece, that it is practically useless for my purposes, and even with the $\frac{1}{12}$-in. it is much too restricted for general use. Can nothing be done to increase the field illuminated?

According to Mr. Nelson and almost all the authorities, you may enlarge your area of illumination by turning the flame of the lamp so that you get a broader area, which may be quite sufficient to fill up the field of a $\frac{1}{12}$-in.; but the use of a bull's-eye is to be avoided in all very critical work.

He says: " For critical work never use a bull's-eye. The edge of the flame is the only critical image; the bull's-eye destroys it.

" The bull's-eye is introduced to make the luminous image large enough to cover an object when shown on a dark ground by means of a stop at the back of an achromatic condenser.

" The only critical condition for a microscopic image is when the object is in the apex of two conjugate cones, and the illuminating rays travel along the same path as the image-forming rays. Now, if the bull's-eye be used and an image of the bull's-eye be formed in the plane of the object, then the image at the back of the objective will be the image of the lamp flame.

" This, when you examine it by removing the eyepiece, may be large enough to fill the back of the objective, but sometimes it will not do so. In brief, it is a different thing altogether from the edge of the flame, which, so far as I know, is the best light for observational purposes. If a bull's-eye be used with the source of the light at its principal focus, then the surface of the bull's-eye becomes the source of light, and must be treated accordingly; that is, it must be focussed on the plane of the object by the sub-stage condenser. The moment you either rack up or down the sub-stage condenser the image ceases to be critical."

The question which naturally occurs is, Does one see more, or see better, with critical illumination or with the intervention of a bull's-eye? Personally, I must admit that I see very little difference between the one and the other, but when I want to get the very most out of an objective, I use critical illumination without the use of the bull's-eye, taking care that the back lens is full of light, which necessitates the slide being of about the correct thickness for the condenser in use.

For photomicrographic work I always use critical illumination without the bull's-eye, except for medium or low powers. One must remember, too, that, although the total amount of light obtained by means of the bull's-eye is greater, the intensity on any given area is less than when the image of the lamp flame itself is brought into focus.

The transition from critical illumination to illumination by means of the bull's-eye, or *vice versa,* takes but a second of time, provided the bull's-eye be fitted as I have described.

The actual method of using the bull's-eye is as follows :—

With the $\frac{1}{3}$-in. or 8 mm. objective and a low eyepiece obtain critical illumination of the object, *i.e.*, the object and the image of the edge of the flame are in focus, and the condenser is central.

1. Turn in the small bull's-eye till the margin of it is central in the field of vision; if it be not central, move the bull's-eye about till it becomes so, but on no account move the mirror or the sub-stage centring screws.

If the bull's-eye be of large size, the margin will not be included in the field, but this might be overcome by having an iris diaphragm fitted to it, as is often done. It is well at this stage to examine the back lens of the objective in the manner so frequently described, when it will be seen that the back lens is now not full of light, but on both sides there is a dark area, and sometimes an image of the flame is seen. (See Figs. C and D, page 40.)

2. Then rack up the condenser slowly till, (*a*) on looking down the eyepiece, an enlarged image of the flame is faintly seen. This is not always quite easy to make out, but closing the diaphragm helps to make it more evident, both with the low and the high powers. (*b*) Examine the back lens, and it will now be found that, if the condenser has been racked up to the right level, the back of the objective will be full of light (Fig. A).

3. Finally, open or close the iris diaphragm till a three-quarter cone of illumination is obtained (Fig. B). This, as was pointed out before, in the case of the $\frac{1}{12}$-in. objective, is when its margins are just appearing (Fig. A).

If it be found that, although the margin of the bull's-eye was perfectly central, the enlarged faint image of the flame is not quite central when the condenser is racked up, it shows that the bull's-eye is not quite at right angles to the rays of light coming from the lamp. This should be corrected, as any deviation of the flame from the centre makes a considerable difference to the results, especially in dark-ground illumination.

To turn from illumination with the bull's-eye to critical illumination one need not take one's eye from the ocular, but merely rotate out the bull's-eye, slightly rack down the condenser till the image of the flame is again sharply in focus, and, if need be, slightly close in the iris diaphragm.

The Condenser and the Thickness of the Slide.

Just as an objective is constructed to give the most perfect image through a cover-glass of given thickness at a given tube-length, so a dry condenser will only give the best results through a slide of a certain thickness with the lamp at a definite distance.

Oil immersion condensers, like oil immersion objectives, are not so affected. When using an oil immersion condenser, if the slip or slide be too thick, it is impossible to focus the edge of the flame; if it is too thin, beyond the fact that the oil will run when the microscope is inclined, a critical image can be obtained.

Most of the modern oil immersion condensers, such as Watson's holoscopic, will work through a slip not exceeding 1·3 to 1·4 mm. thick, when the lamp is placed somewhat close to the microscope. Others, such as Beck's, will work through a slide up to 1·9 to 2 mm.

If with either of these condensers a thin slide, say, of 0·9 mm. thick, be used, the oil will be constantly running and breaking oil contact. To obviate this Nelson uses a slip equaliser. This is a piece of cover-glass of varying thickness, the most useful being 5 mm. and about 1 in. square, with a piece of glass $\frac{1}{8}$ in. wide cemented along one side. This is oiled to the back of the slip, so that the ledge prevents it slipping down.

How to determine the correct thickness of a slide when used with a **dry condenser** and the **lamp at a fixed distance.**

1. Place on the stage a very thin slide, say, of tubercle bacillus or *S. pallida.* With a $\frac{1}{3}$-in. objective focus the organisms and the edge of the flame. Open the iris diaphragm fully. Disregard for a moment the organism and observe the image of the flame.

It will be found that (i.) the image of the flame is ill-defined, (ii.) on racking down the condenser slightly the flame image will become misty and its margins rapidly disappear; on racking up the condenser beyond its focus the reverse takes place, and the flame image remains hard and its margins sharp.

This test can be modified and, I think, improved by the following :—

2. Focus the object and the image of the edge of the flame, instead of racking the condenser up or down, use the fine adjustment and focus slightly up and down. The results will be precisely the opposite of the above, if the flame image becomes misty on focussing up, but its margins remain hard and sharp on focussing down, the slide is too thin. If the slide be too thick, the results in both cases will be the reverse.

It is much easier to use the fine adjustment of the microscope than the rack and pinion of the condenser, but it is advisable to use this method carefully with a high-power objective as in focussing down one may touch the cover-glass with the front lens; this, however, is not a very serious matter if the fine adjustment is being used. In order to assist the memory, I find the following the best rule when testing, at the same time for correct tube-length for cover-glass thickness and the correct thickness of the slide, using the fine adjustment, the iris fully open and a critical image of the flame :—

" Rings downwards, lengthen the tube; flame sharp downwards, strengthen the slide."

3. If the eyepiece be removed and the back of the lens be examined, particularly when the $\frac{1}{12}$-in. is in use, it will be found that when the slide is too thin, on racking up the condenser above its focus, a black patch appears on either side of the centre. (See Fig. C, p. 40.) If the slide be too thick, the black patches appear when the condenser is racked down.

The correct thickness of a slide is found when the appearances are equal in either of the above cases, whether the condenser be racked up or down, and the image of the flame appears sharply defined when it is exactly in focus under any objective, even when the iris diaphragm is widely open.

The reason of the above is, that when a slide which is too thin is used, the condenser is under-corrected and the marginal rays come to a focus nearer the front lens than those passing through the centre, whilst, when the slip is too thick, the condenser is over-corrected and the marginal rays come to a focus further from the front lens than those of the centre.

How to correct under-correction of the **condenser** due to the slide **being too thin.**

This is by far the most common condition.

(i.) The lamp may be moved further off, but in our case it is fixed to the microscope board.

(ii.) Unscrew the top lens of the condenser till the image of the flame is equal above and below the focus. This succeeds in most cases, and it would be a great advantage if makers would fit a collar-correction to condensers, as is the case with some objectives. I have seen some apochromatic sub-stage condensers with this adjustment. In the case of Watson's parachromatic condenser, which I have in daily use, I find that the maximum thickness of a slide through which the condenser will work is about 2 mm., and the optimum 1·6 mm.

By unscrewing the top lens I can correct it for any slide down to 0·8 mm. thick, and lately I have had three washers made in brass of different thickness, one of which I place on the condenser after the top lens has been removed, and then screw up the latter again in its place.

(iii.) Another, and a very simple method of correcting the thickness of the slide, is to apply varying thicknesses of cover or other thin glass by means of a drop of cedar oil to the under surface of the slide.

Recently I received a very valuable set of blood films from the Rockefeller Institute, spread on exceedingly thin slides, varying from 0·65 to 0·9 mm. In this case, for temporary use, I placed a drop of cedar oil on the centre of another slip of about 0·9 to 0·7 mm. thick, and placed them together. This method has the advantage that oil does not get on to the surface of the stage as readily as it would do were a cover-glass applied.

Afterwards, the two slides were fixed together by Canada balsam, making a slide of, approximately, the correct thickness. The only disadvantage of this method is that the position of an object as "logged" may not be quite correct.

The disadvantage of an incorrect thickness of the slide is appreciable visually and, even more so, photographically, but it is not nearly so important, in my opinion, as the question of tube-length and cover-glass thickness.

"You make a great deal of slip thickness, much of which is hypercritical. The real thing is, that condensers are very faulty optical instruments. In other words, optical corrections are limited to a certain amount of Optical Index (O.I.).

"Consider the facts, a ½-in. objective of N.A. 0·65 is a tremendous achievement, and requires a fluorite apochromat for its attainment. But what shall we say of a condenser of ½-in. with N.A. 1·2, and not a scrap of fluorite in it. Of course, it is full of uncorrected aberrations."—*Nelson.*

Take a thin slide, say, 0·9 mm. thick, of *S. pallida*, and examine with the ½-in. objective, dry condenser with full cone, and critical image of the edge of the flame; focus a spirochæte and see how much you can resolve with different eyepieces, using, of course, the correct tube-length.

Then remove the eyepiece and examine the back of the lens, and you will see that the area of illumination is elliptical in shape. Remove the slide and apply a cover-glass of about 0·6 mm. in thickness to the under surface of the slide by means of a drop of cedar oil. Replace the slide and examine the same spirochæte as before under critical illumination, and you will notice that (i.) there is much more light and the lens will stand a higher eyepiece, (ii.) you will see details that you could not see, or saw indifferently before, much more clearly, and (iii.) you will find the back lens is like a full moon, round and, apparently, larger.

Photographically there will be a decided improvement when the slide is of the correct thickness. For some years I have been taking twelve to sixteen photographs on one quarter plate; the subjects may have been the same, but they were not taken from the same slide, perhaps five from one, three from another, or only one from each slide. Although all were taken at the same time under apparently precisely the same conditions, I found the results were very different—some were very under-exposed, some slightly so, others perfect. I could not detect the cause of this for a long time, until at last I noticed that the very under-exposed photomicrographs were all from too thin slides.

I should certainly say that a very thin slide would require about double the time of exposure that a slide of correct thickness would if the magnification were 1,000 diameters.

If the slide be too thick, practically nothing can be done, except to bring the lamp, if it is not fixed, as near as possible to the back lens of the condenser.

Dark-ground Illumination.

This is a method in which the direct rays from an illuminant are not allowed to enter the objective, so that the object appears luminous on a black or dark ground.

It may be attained by placing a central stop at the back of a condenser, which may be used dry or immersed, or by means of a special illuminator used immersed, in which a permanent stop is fixed at the back of a specially constructed condenser.

It is obvious that the condenser must have a greater numerical aperture than the objective, and the stop must be of such a size as to cut out all rays of less N.A. than that of the objective.

An achromatic condenser, used dry or immersed, *i.e.*, in oil contact with the under surface of the slide, in conjunction with a stop which can be rotated in or out of its position at the back of the condenser, has one great advantage over that of the special dark-ground illuminator, namely, it can be used for ordinary illumination or for dark-ground illumination at will.

A dry condenser with a suitable stop will give dark-ground illumination with objectives not exceeding N.A. 0·65.

An oil immersion condenser with a stop may be used with objectives of somewhat larger N.A., possibly even up to 0·7 or 0·8, or higher. I may mention here that the best description of dark-ground illumination in all its aspects is one given by Mr. M. A. Ainslie, B.A., entitled "Dark-ground Illumination in Microscopical Work," in the *Journal of the Royal Army Medical Corps*, 1913, vol. xx., No. 4, p. 379.

Method of obtaining Dark-ground Illumination with a Dry Condenser.

Place on the microscope a ⅓-in. of N.A. 0·65, or any other objective with an N.A. not above that amount, the lowest eyepiece, say, No. 0 or 1, and in the sub-stage an achromatic condenser, preferably the small type, such as Watson's parachromatic. Prepare a slide of any living organisms, say, the spirochætes from the teeth. Scrape a decayed tooth with a toothpick, and place the speck of material so obtained on a slide, and cover with a cover-glass.

1. Proceed as described for obtaining critical illumination, *i.e.*, focus the object with any light, focus the edge of the flame, and centre the condenser so that one obtains a field like A.

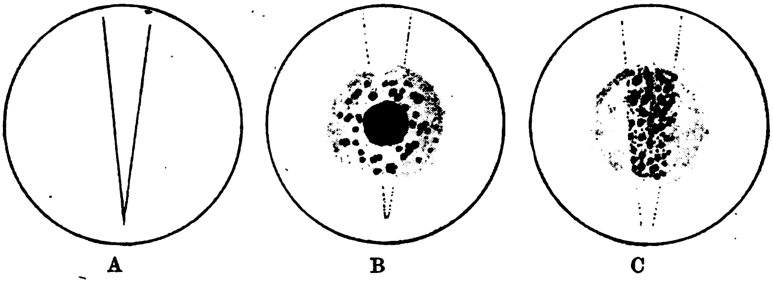


Diagram of Stages in obtaining Dark-ground Illumination.

2. Remove the colour screen, if there be one, and open the iris diaphragm fully. By means of the stop carrier rotate in a small stop, or, better, use a Traviss' expanding stop closed to such a size that it is a little wider than the width of the flame. The effect will be as at B.

It may happen that this blurred image of the stop is not quite central; it should be central with the iris diaphragm. Centre the stop by means of the centring screws of the sub-stage, and replace the image of the lamp flame central to this by slightly tilting the mirror. What you probably now see is that the top and bottom of the image of the flame are unaltered, but in the centre of the field there is a blurred dark area which is blacker in the centre as at B.

If the stop is too small or too large, open or shut the expanding stop, or, when using the movable stops, replace it by a larger or smaller size as may be necessary.

3. Rack up the condenser very gently, watching the effect, till the central portion of the blurred area shows a small black ground in which the organisms are brilliantly illuminated, as C.

In probably 90 per cent. of cases it is necessary to slightly rack the condenser upwards, in a few cases there may be no need to move the condenser either upwards or downwards, whilst in the remaining few it may be necessary to rack the condenser slightly downwards before the above effect is obtained. The reason of this is that, in the majority of cases, the slide is too thin for the condenser to work at its best, and there is spherical aberration and under-correction. In a few cases the slide is just the right thickness, whilst in the third case there is over-correction, as the slide is too thick and the condenser requires to be racked down.

4. Replace the lowest eyepiece by a higher eyepiece, say, comp. oc. 8, or the ordinary No. 4, and it will be seen that you have considerably magnified the brilliant black-ground area, and it is advisable at this stage to try whether the brilliancy of the objects in the dark-ground area is increased by slightly racking the condenser upwards or downwards, or by opening or closing the expanding stop.

As a rule the more the stop is enlarged, the more the condenser will need racking up. This is a measure of its spherical aberration.

It is very generally stated that a given condenser requires a definite size of the stop when used with a given objective, but, as a matter of fact, I find that the size of the stop varies with the power of the eyepiece in use.

As you increase the power of the eyepiece so, in my experience, you require to diminish the diameter of the stop in order to get the best results. The stop that gives the best picture with comp. oc. 8 will not give the most brilliant result with comp. oc. 18.

It is well to remember (i.) that as far as possible the condenser should not be moved much from the plane of its focus,* otherwise the image of the flame becomes much less brilliant ; (ii.) that very slight alterations in the size of the stop make an enormous difference in the result ; (iii.) that if the centre of the dark-ground area be not brilliant, but of a dark cloudy appearance, it means that the condenser must be racked up or down till the central area is very luminous ; and (iv.) that it is well to work the size of the stop against the level of the condenser, *i.e.*, to slightly vary the size of the stop and, at the same time, to rack the condenser up or down till the most brilliant image is seen.

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* Note it has a plane of focus for each sized stop, because of its spherical aberration, and so, when you increase the size of the stop you have to rack up, because the flame image is no longer in focus. (Nelson.)

For these reasons, it is infinitely easier to use an expanding stop than various sizes of separate stops, as one can, in the former case, so readily see and control the effects of the slightest alteration in the stop.

5. When you have obtained a dark-ground effect by this means you will, in all probability, be very disappointed with the image, therefore the next thing is to correct the tube-length in the manner previously described, and you will obtain a good dark ground, but of small area.

6. **The use of** the **bull's-eye** with **dark-ground illumination.**—Having obtained a small, but brilliant dark ground, remove the high eyepiece and place the lowest in the tube, and rotate in the bull's-eye, and when this is central it will be seen that the stop must be considerably enlarged, and the condenser racked up. By increasing the size of the stop and by raising the condenser, a point is reached at which the best and most brilliant image is obtained.

The one place to watch, in order to get this, is the centre of the field, if that is dark or dull the condenser is either racked up too much or not enough, or the stop is too large.

I have always placed the bull's-eye condenser at the same distance from the flame, *i.e.*, at its focal distance, for ordinary and for dark-ground illumination.

Mr. Nelson suggested that a more brilliant dark-ground effect could be attained by placing it as near the lamp as possible. I have tried this and find that it is a decided improvement.

Dark-ground illumination obtained by means of a dry achromatic condenser with an expanding stop, and in conjunction with the bull's-eye, is, in my opinion, the most practical and most convenient of all the methods, and I use it almost daily. Its advantages are :—

1. You use the same condenser as you do for ordinary work.

2. You are not concerned very much about the thickness of the slide, it will work with a slide varying from the thinnest to one of 1·8 or 1·9 mm. thick.

3. You have not the bother of oiling the slide to the condenser.

4. You can pass from ordinary to dark-ground illumination in a moment or two, without taking your eye from the eyepiece.

Its disadvantages are that it will not work with an objective exceeding N.A. 0·65, and it does not give quite such a brilliant result as that obtained by means of an oil immersion condenser or a special illuminator used oiled.

The best test of the relative brilliancy of a dark ground is a slide of living *Spirochæta pallida*, and if such can be obtained on a slide, not exceeding a thickness of about 1·1 mm., the three methods of dark-ground illumination, viz., by means of (*a*) a dry condenser and stop with the bull's-eye, (*b*) an oil immersion condenser and stop, and (*c*) a special illuminator, can be compared. It will be found that the most brilliant, with an ordinary oil lamp as the illuminant, is that obtained by means of an oil immersion condenser and stop, and probably the dry condenser with stop and bull's-eye will be the least brilliant, but by far the most convenient. The brilliancy of the illumination, whether it be ordinary transmitted light or dark-ground illumination, is relative. If the room be flooded with sunlight or even electric light, I find that it is well-nigh impossible to see the delicate spirochætes with either form of dark-ground illumination, but if the room be dark any or all become quite brilliant.

Dark-ground Illumination with an Oil Immersion Condenser.

For this purpose I find the beautifully corrected holoscopic oil immersion condenser of Watson by far the best.

The first thing is to test what is the maximum thickness of a slide through which the condenser will work when oiled, with the lamp at the distance we have assumed.

Take a few slides of a thickness varying from 1 to 1·4 mm., place a drop of red ink or stain in the centre and apply a cover-glass. On the top of the condenser place a largish drop of cedar oil and, placing the slide on the stage, focus the particles of colour and rack up the condenser till the image of the edge of the flame is exactly in focus. When it is found that the slide is slightly lifted by the condenser, one knows that it is too thick.

I find that my holoscopic oil condenser will not work through a slide exceeding 1·3 mm.

Take a film, say, of *Spirochæta pallida*, or of the spirochætes of the mouth on a slide of, say, 1·2 mm. thick. Oil this to the condenser. Focus any object with the ⅓-in. objective, rack up the condenser till the flame image is precisely in focus. Centre the condenser by (*a*) closing the iris diaphragm and racking up the objective by the coarse adjustment till the small opening in the iris is seen, and bring that opening central by the sub-stage centring screws; or (*b*) rotate in a small stop till it is quite central and lies over the image of the flame. Expand the stop till it is a little larger than the width of the flame, and it will be seen that objects are brilliant on a small dark ground in the centre of the field. Replace the low eyepiece by the comp. oc. 18, and there is a very brilliant dark ground in the area of the flame image. This can be varied by slightly altering the size of the stop and, perhaps, by very slightly raising the condenser.

Lastly, correct the tube-length for the thickness of the cover-glass. It will be seen that the method of obtaining a dark ground with an oil immersion condenser is precisely like that described for the dry condenser, with the exception that with the former it is hardly ever necessary to rack up the condenser, whilst with the latter it is almost the rule.

In my experience the oil immersion condenser gives the best dark ground without a bull's-eye, whilst the dry condenser generally requires one.

The size of the dark ground with the oil immersion condenser is small, but it can be somewhat increased by slightly turning the flame of the lamp round and also slightly expanding the stop. In this way one gets a larger dark ground, but one of less brilliancy. Dark-ground illumination obtained by means of an oil immerson condenser and stop has the advantage that it gives the most brilliant dark ground of all, that it is the easiest to use of all the immersion condensers, and lastly, one can pass from dark ground to ordinary illumination in a moment by swinging out the stop.

As Mr. Nelson says, when writing on the special dark-ground illuminators : " With regard to all these new kinds, or rather old kinds revived, of parabolic and what not, it was my opinion when formerly they were in vogue that none of them could compare with an oil immersion condenser, and that remains my opinion still."

It has the disadvantages (i.) that slides can only be used up to a certain thickness, in the case of my holoscopic condenser not exceeding 1·3 mm. ; (ii.) that too thin slides have to be built up with cover-glasses or the slip equaliser used, otherwise the oil runs and oil contact is broken ; (iii.) that the use of any form of oil immersion condenser is rather a nuisance, especially on the ordinary mechanical stage, as the oil tends to run on to the surface of the stage if the slide be moved about much ; (iv.) that the field of dark ground is limited to the width of the flame and is rather restricted when a low eyepiece is used for searching for an object. This, however, may be enlarged at the cost of brilliancy by slightly rotating the flame, or even by the use of a bull's-eye, provided that the slide is thin enough to allow the condenser to be racked up a little further.

On the whole, I am decidedly in favour of not using the bull's-eye with this form of illumination, whilst with dark ground obtained by the dry condenser the use of a bull's-eye greatly improves the resulting picture, and is almost a necessity.

Dark-ground Illumination by means of Special Condenser.

Condensers specially constructed with fixed stops have been introduced, or, rather, re-introduced, lately for giving dark-ground illumination. They are all used immersed, and, unlike the achromatic condensers described above, they can only be used for dark-ground effects. These vary in their form and construction.

In England they are made by Watson, Baker, Beck, Swift, etc., and on the Continent, Zeiss, Leitz, Reichert, etc.

Nelson's dark-ground illuminator, made by Baker, is said by Ainslie to be "the most effective dark-ground illuminator, and in the writer's hands it has given, perhaps, the best results of any of the special forms."

For a considerable time I have used a Leitz illuminator, and prefer it to any of the forms that I have tried.

All the makers supply instructions with their instrument, and I will only briefly allude to the more important points in the method of using them. I fancy that all work through a slide about 1 mm. thick, and the Leitz model about 1·1 mm., when the lamp is near the microscope. If the slide be above that thickness, it is impossible to use it; on the other hand, if the slide be so thin that the oil breaks contact, then it may be built up by means of a cover-glass in oil contact. The only difficulty with these illuminators is the centring.

Place the dark-ground illuminator in the sub-stage and rack it up till the top of it is level with the surface of the stage.

Use a ⅔-in. objective and a low eyepiece, and focus the small rings engraved on the surface of the condenser; these are not always quite easy to see, but a slight movement of the mirror or bull's-eye will help to show them. Centre these circles to the field of vision. Then rack down the condenser, place a large drop of cedar oil, glycerine or water on the top of the illuminator, and put the slide on the stage. Then rack up the condenser till the oil comes in contact with the slide.

Focus the object with the same objective, so that a small area of light is seen. Put the bull's-eye in position, and so enlarge the luminous area, and rack up or down the condenser till you have obtained the smallest, but the most brilliant, area.

Replace the ⅔-in. by the ⅓-in., and if the area of light is not central, put it in the centre by means of the centring screws of the sub-stage, but do not move the mirror.

Focus the object very carefully, and rack the condenser very slightly up or down until the most brilliant results are obtained.

It is well to remember the following rules, whatever be the means by which dark-ground illumination is obtained :—

Firstly, that the very slightest movement of the condenser upwards or downwards makes an enormous difference in the brilliancy of the image, and for this form of illumination I can quite imagine that a fine adjustment to the sub-stage might be a very valuable adjunct.

Secondly, to determine the very best position of the condenser, watch carefully the centre of the luminous area ; if this be darker than its surroundings, the condenser is either racked up too far or not far enough, and one can determine which by a slight movement of the sub-stage. If the central area be dark and the condition is made worse by racking downwards, then rack the condenser upwards ; but if in the latter case, although some improvement is seen, on racking further up the object goes out of focus, you know at once that the slide is too thick.

If the lamp be movable, bringing it a little closer may help ; if not, the slide must be discarded as hopeless.

In all methods of dark-ground illumination the greatest care should be taken that the slides, and particularly the cover-glasses, are scrupulously clean, and when the immersion condensers are used that there are no air-bubbles in the oil.

Water may be used instead of oil for immersing the illuminators, as mentioned by Leitz in his directions for the use of his dark-ground condenser, but this is only true when the organisms are in a watery or serous medium, not when they are mounted in a medium of higher refractive index, such as Canada balsam or liquid paraffin.

Various illuminants may be used with dark-ground illuminators, and I have used, especially on living specimens, a Nernst light. Many other workers use a small arc light or the Pointolite lamp made by the Ediswan Company. I have tried an acetylene bicycle lamp, but I find it unreliable and not easily controlled.

For all-round dark-ground work I prefer a dry achromatic condenser, and for special purposes an oil immersion condenser with a stop, both being used with an ordinary microscope lamp.

One point to be remembered is that, the more intense the illuminant, the more brilliantly do all the little particles floating in the medium of living preparations become evident, obscuring to a certain degree the organism which is under examination. With a less powerful light, while the organism itself is certainly not so brilliant, yet it may be even more readily seen, as the surrounding refractile atoms are very much less in evidence.

As to what objectives are most suitable for dark-ground illumination may be a debatable question. At least, all will agree that the objective must be a good one, especially in the case of a dry lens, as it is put to a very severe test by this method of illumination, and if it be only of a power of the 8 mm. or ⅓-in., it will commonly have to be used with the highest eyepiece, the comp. oc. 18, which will further test its quality.

I have used for many years Zeiss's and Leitz's apo. 8 mm. or ⅓-in., of N.A. 0·65, and I want nothing better, as these lenses both stand the highest eyepiece well, and yet with the lowest oculars, No. 0 or 1, they can be used for centring the condenser and getting everything in perfect adjustment.

A $\frac{1}{12}$-in. objective can be used, provided its N.A. is cut down by means of a stop to about 0·9 for the special illuminators. Leitz supplies a funnel stop for cutting down the aperture of their immersion lenses, and probably other makers do the same; but, of course, the funnel stop has to be removed when the objective is used for ordinary observational purposes with transmitted light.

In order to overcome this difficulty I am using a Traviss' iris diaphragm fitted in the mount of the objective, so that I can reduce the aperture of the lens even to N.A. 0·65, and by this means can get a fairly good dark ground even with a dry condenser with a stop, and, without removing the objective, I can pass to ordinary transmitted illumination by opening the iris of the objective and also removing the stop at the back of the condenser.

The Traviss' iris in the objective does not give quite such a good dark ground as the funnel stop does, probably because the former does not come as close to the back lens of the objective as the latter.

Does a $\frac{1}{12}$-in. oil immersion with a suitable stop give a better dark ground than a dry lens, say, the $\frac{1}{3}$-in., without a stop? This is largely a matter of opinion. Those in favour of the use of an immersion objective fitted with a stop maintain that these lenses are much less sensitive to variations in the thickness of the cover-glass; that there is no reflection from the surface of the objective; that the very best part of the lens is being used, as its margins are stopped out, and that higher magnification can be obtained.

Personally, I prefer for general use a dry lens, especially the 8 mm. apo., as corrections of the tube-length for cover-glass thickness can be made in a few seconds.

The Author's Method of Dark-ground Illumination. On Stained but Unmounted Preparations.

Very little mention is made in any of the text-books of the value of dark-ground illumination on stained preparations.

Since 1910 I have been using a method which has proved invaluable for the detection of spirochætes, especially *Spirochæta pallida*, *Leptospira icterohæmorrhagiæ* (jaundice), *Leptospira icteroides* (yellow fever), and the spirochætes of relapsing fever.

I first described this in 1914, in *Parasitology* ("Blood Parasites Found in Mammals, Birds and Fishes in England"), and more fully in the *British Medical Journal*, 1915 ("An Easy Method of Detecting *Spirochæta pallida* and Other Spirochætes").

I have quoted here very largely from the latter communication.

"The following method which I have devised renders the detection of *S. pallida* or any other spirochæte as easy as that of the tubercle bacillus in sputum. It depends on the following principles:—

"1. That structures which are coloured with a fluorescent dye—for example, eosin, fuchsin, fluorescin, etc.—when examined on a dark ground, stand out very prominently, while blue-stained structures are very inconspicuous.

" 2. According to Bolles Lee, ' the visibility of minute colourless structures is proportional to the difference between the refractive index of the object and of the medium in which it is mounted.'

" Assuming the R.I. of bacteria is, as A. Fischer states, 1·55, that of solid Canada balsam 1·538, balsam in xylol a little lower, say, 1·53, liquid paraffin or parolein 1·471, distilled water 1·336, and that of air 1·0, an organism, stained or unstained, would be more easily seen in air than in either of the other media.

" Therefore, if the medium in which the red-stained organisms lie be of very low refractive index, they are exceedingly easily detected with a comparatively low power, but if mounted in balsam, cedar oil, parolein or water, they are only seen with the very greatest difficulty.

" The method, briefly, consists in examining a film containing spirochætes stained in the usual way with Giemsa or Leishman's stain dry, not mounted in any medium, with dark-ground illumination, using a dry lens of medium power.

" There are, however, some details which considerably simplify this problem.

" Stain a film, say, of *S. pallida*, with Giemsa for any period from one minute to twenty-four hours, preferably not less than ten to fifteen minutes ; wash, dry, but do not mount, and examine it by dark-ground illumination.

" **Dark-ground Illumination.**—The special dark-ground condensers in use—for example, those of Leitz, Zeiss, Baker, etc.—have two disadvantages for this examination, as, first, they all require to be in oil contact with the under surface of the slide, with the result that one cannot examine a large area of the film without the oil running on to the surface of the stage or its being used up ; and, secondly, they do not allow the change from dark-ground to ordinary illumination being made. To overcome these disadvantages, and to do away with the necessity of using oil, I use an ordinary achromatic condenser, used dry, without oil, provided with a Traviss' expanding stop below it. A simple round stop, as long as it be of the right size, could be used ; but the expanding stop is much more convenient. Any of the microscopic agents would fit either of these, and, if necessary, explain how a dark ground is obtained. An ordinary paraffin microscopic lamp, used with or without a bull's-eye condenser, is ample illumination.

"The objective I use is an 8 mm. or $\frac{1}{3}$-in., of Zeiss and Leitz, and a compensating ocular of 8, 12, or 18, or an ordinary No. 4 or 5 eyepiece. I can see *S. pallida* with a $\frac{2}{3}$-in. objective with 18 ocular, but I could not pick them out with this low power. I find I cannot use a $\frac{1}{6}$-in. objective on an uncovered objective, and its N.A. is too large to obtain a good dark ground with this method.

"The stained film must not be mounted in balsam, oil, water, or any other medium; it must be examined dry in air. The easiest and best result is obtained by examining it without a cover-glass, but if such an uncovered film be examined with a dry lens at the correct tube-length of that lens for a covered object, the image will be very imperfect and practically useless, but if the tube of the microscope be lengthened to the right height—this can only be found by trial, but, when found, will be practically constant for that objective—the definition is perfect.

"My Zeiss 8 mm., normally working at a tube-length of 160 mm., requires to be lengthened to about 195 mm., and the Leitz 8 mm. objective from 170 mm. to 240 mm. The latter length cannot be obtained on an ordinary short-tube microscope without the addition of a cardboard tube. I have adjusted the latter since, so that now the objective is perfectly corrected for an uncovered object when the draw-tube of my microscope is drawn fully out—that is, to a tube-mark of 192 or a tube-length of 210 mm. Of course, the inconvenience of lengthening the tube may be overcome by laying a cover-glass on the film, when the objective will be used at about the normal tube-length and the definition will be excellent. This, however, has the disadvantage that, if a spirochæte be found by dark-ground illumination, and one wishes to examine it with ordinary illumination by removing the stop under the condenser and turning on the $\frac{1}{12}$ oil immersion lens, the spirochæte will not be seen. On the other hand, if there is no cover-glass, the $\frac{1}{12}$ lens oiled will enable it to be seen at once.

" In a stained film examined on a dark ground without any mounting medium, S. *pallida* can be easily detected with such a moderate power as a $\frac{1}{3}$-in. objective. It stands out as a beautiful golden spiral, which cannot be missed by the merest tyro.

" The large field of vision, the ease with which a slide can be thoroughly examined, the absence of any eye strain, and, above all, the remarkable way in which the spirochæte strikes the eye, have only to be seen to be appreciated.

" I have now used this method for some years, and am convinced that I should find no difficulty in finding a spirochæte, even if only two or three were present in a film the size of a postage stamp. Can the same be said of any other method now in vogue for the detection of these illusive organisms ?

" It seems to me that it would be invaluable to those who are searching for S. *pallida* in films from the brain in cases of general paralysis of the insane, etc.

" It is, of course, equally serviceable in examining the blood for any of the spirochætes, provided these are coloured a reddish tint with Giemsa, or if stained with eosin or fuchsin. Blue-stained objects are very inconspicuous.

" Some few years ago, whilst examining a Giemsa-stained film with the $\frac{1}{12}$ oil immersion lens, I found a spirochæte in the blood of a bat, which I provisionally named S. *vesperuginis*. This spirochæte, very slightly larger than the S. *pallida*, was only present in extremely small numbers. It would have been well-nigh impossible to have found another with the $\frac{1}{12}$-in. objective, but in the remaining fifteen films, which were not mounted, I had no difficulty in detecting and marking these spirochætes by examining the films dry on a dark ground, although in many films there was only a single spirochæte present."

In order to put to the test the previous statements, take a stained, but unmounted dry film containing S. *pallida*, or even the spirochætes of the mouth.

Examine it by dark-ground illumination by any of the methods previously described, preferably with the dark-ground obtained by means of a dry achromatic condenser and stop used in conjunction with the bull's-eye.

Use an 8 mm. or $\frac{1}{3}$-in. apo. objective and comp. oc. 18. Correct the tube-length for an uncovered object by pulling out the draw-tube to its fullest extent, say, 210 mm.

Find a *S. pallida*, notice how brilliantly it stands out with all its spirals so clearly seen, provided the tube-length be correct. Then, without altering the above conditions, push in the draw-tube to its lowest position; on focussing, the whole field is seen to be hazy and milky, and the spirochæte appears very indefinite and not easily recognisable.

Correct the tube-length till the image is again perfect, and then try the effects of a lower eyepiece, say, comp. oc. 8, or ordinary ocular No. 4, and again notice how beautifully the spirochæte stands out, sharply, clearly, and conspicuously, even with this magnification, which is probably about 320 diameters. Try a lower eyepiece, say, comp. oc. 6, or ordinary ocular No. 3, which probably gives a magnification of 240 diameters at this length of tube, and note how easily the organism can be found.

Mark the position of a good spirochæte by drawing a very small ring round it with the diamond marker, or by logging its position on the stage. Remove the slide and mount the preparation in Canada balsam or liquid paraffin, and replace in the same position on the stage, and look for the ring and spirochæte. Correct the tube-length now that the film is mounted by lowering the draw-tube to, say, the mark 160, and you will find that the spirochæte is not nearly so prominent, but when seen at the correct tube-length is still a very beautiful object.

Keeping the slide in position, remove the stop from behind the condenser, put in position the blue screen, turn on the $\frac{1}{12}$-in. objective, and examine the field with ordinary transmitted light. It will be noticed that the spirochæte is not at all easily found, especially if the film has been lightly stained, and it could be very easily overlooked in a rapid survey.

I find that if a film containing *S. pallida* be stained by Giemsa for sixty seconds by the watch, the spirochætes are easily detected by dark-ground illumination obtained by means of a dry condenser, but that when the $\frac{1}{12}$-in. objective is put on, after removing the stop, and the preparation examined by ordinary illumination, the spirochætes are extremely difficult to see, but if found, they appear much more delicate and beautiful than when deeply stained.

Are all spirochætes more easily found on an unmounted than on a mounted Giemsa stained film by dark-ground illumination ?

If the preparation be a blood film, yes, but some films contain so much granular material that the spirochætes are somewhat masked by the refractile granules.

For example, S. *icterohæmorrhagiæ* in a stained blood film are more easily seen when unmounted, but when the spirochætes are in the granular debris from the kidney I think they are more evident when the slide is mounted, or when a drop of liquid paraffin has been placed on the slide and allowed to drain off. In the latter case no cover-glass is used, and the tube-length will be the same as for an uncovered object, and this procedure has the advantage that a rare spirochæte or other object can be marked by means of the diamond object marker, before the preparation is finally mounted.

Take another example, tubercle bacilli in sputum stained in the usual way by Ziehl-Neelsen's method, in which the T. bacilli are red on a blue background. Mount one-half of the film in Canada balsam.

Examine the mounted end with dry dark-ground, using the 8 mm. or ⅓-in. apo. and comp. oc. 8, and the tubercle bacilli are beautifully seen as tiny golden rods on a blackish background.

Now examine the unmounted end under the same conditions, except that the draw-tube is pulled out to the correct length, and you will find that the whole field is much too granular to detect any bacilli. Smear over this end of the film a little liquid paraffin and the bacilli are well seen.

Micrometry.

The first essential is a good **Stage micrometer** and, undoubtedly, the very best is that ruled by Grayson and sold by R. and J. Beck.

The rulings are the inch divided into 100th and 1,000th, or the millimetre divided in 0·25 or 250 μ; 0·1 or 100 μ; and 0·01 or

10 μ. The English and metric rulings can be obtained on separate slides, or both mounted on the same slide.

I have them both mounted under the same cover-glass, and I find it is not very difficult to get confused as to which happens to be in the field, therefore, I advise that they be obtained separately mounted; if only one, certainly the metric ruling should be chosen.

The next is the **Eyepiece micrometer,** which is a small disc of glass with divisions (5 mm. divided into $\frac{1}{10}$ mm.), ruled on its under side. That made by Winkel is, according to Nelson, the best.

This is placed on the diaphragm of an ocular in such a way that the figures are erect, and the lines are carefully brought into focus by unscrewing the eye-lens. This can be most easily done when the eyepiece is in the microscope tube and a moderate light used. Zeiss, Leitz, and others make an ordinary No. 2, or a compensating No. 6 ocular, which is constructed to unscrew in the centre, in order to facilitate the placing of the micrometer on the diaphragm, and which is fitted with a sliding eye-lens in order to permit of the exact focussing of the divisions of the micrometer. But these, although convenient, are not essential, as the micrometer can be dropped into most of the eyepieces, so that it rests on the diaphragm, where, if it be not in exact focus, the diaphragm may be slightly altered in its position till the marks are clearly seen.

It should be particularly noted that those eyepiece micrometers which have no figures on the ruling should always be placed with the same surface upwards, otherwise the readings will be inaccurate.

It will be necessary to determine the value of eyepiece divisions for each objective at a specified tube-length.

In order to do this, place the stage micrometer on the stage and eyepiece micrometer in position in the ocular, focus the lines on the former, and count the number of intervals of the stage micrometer which overlap any convenient number of intervals of the eyepiece micrometer. It is advisable to make the micrometer value an integral number, otherwise there is a certain amount of calculation to be done each time measurements are made, and I pay more attention to this than to any little departure from the correct tube-length.

Unfortunately, no eyepiece micrometer which I have come across gives a whole number for the $\frac{1}{12}$-in. achromatic lens.

I use two of Zeiss' micrometer oculars, the ordinary or Huyghenian No. 2, and the compensating ocular 6, and the following table shows the value of one eyepiece division with my particular objectives :—

	Ordinary Ocular 2.			Compensating Ocular 6.		
Objective.	Tube mark.	Eyepiece = Stage Divisions.	Value.	Tube Mark.	Eyepiece = Stage Divisions.	Value.
$\frac{2}{3}$-in.	158½	50=750	1=15 μ	152	50=800	1=16 μ
8 mm.	155	50=350	1= 7 μ			
	186	50=300	1= 6 μ	136½	50=400	1= 8 μ
$\frac{1}{10}$-in.	172	50=100	1= 2 μ	192	50=100	1= 2 μ
$\frac{1}{12}$-in.	170	49= 70	$1=\frac{10}{7}$ μ	149	30= 50	$1=\frac{10}{6}$ μ

According to Zeiss, a mere change in the position of the eye-lens of the ocular for the purpose of focussing the scale in the micrometer eyepiece does not affect the reading.

The only real difficulty in using this method of measuring an object is the difficulty of bringing the object in exact alignment with the divisions in the eyepiece micrometer, especially when using a high power. For the long tube microscope, the Jackson eyepiece micrometer, in which a scale is moved by a fine screw across the field, is a very great convenience, and I hope opticians will make a similar one for the smaller oculars of the short tube microscope.

Since this was written, I find that Leitz for some years past has listed a micrometer eyepiece with a movable scale. This should prove of great convenience.

For more exact measuring, the elaborate and somewhat expensive screw micrometer eyepiece is to be recommended.

Description of the instrument and its method of use will be found in the larger text-books, such as Carpenter's or Spitta's book.

It is only possible to measure by eyepiece micrometry the size of an object which is straight. A curved object, such as a spirochæte or a trypanosome, must be either drawn by the camera lucida, projected by means of a projection apparatus, or reproduced by photomicrography before its size can be determined.

Of all methods of micrometry the most accurate is; undoubtedly, that performed by photomicrography.

Method of Measuring the Magnifying Power of a Microscope.

There are various means of ascertaining the combined magnifying power of the objective in conjunction with the eyepiece, and quite recently Oxley has given an excellent account and criticism of these in the *English Mechanic*, June 13th and 27th, 1919.

Briefly, the following are some of the usual methods :—

First Method. Project the image of the stage micrometer by means of a photomicrographic camera on to the ground glass, placed at a distance of 10 in. This distance must be measured, not from the top surface of the ocular, but from the position of the Ramsden disc, which is found by holding a piece of ground glass over the eyepiece ; the distance from the eyepiece to the ground glass when the disc is in focus is added to the 10 in.

Second Method. By means of a Beale's neutral-tinted camera lucida project the image of the stage micrometer on to the surface of a paper, which must be placed precisely 10 in. from the centre of the eyepiece, when the microscope is in a perfectly horizontal position. Oxley states that the 10 in. " should be measured from the centre of the eye-lens in a plumb line to the paper.

" I have found by experiment that a distance of $\frac{1}{20}$th of an inch, more or less, makes a difference of $2\frac{1}{2}$ per cent. in the measurement." Unfortunately, this type of camera, which is by far the best for the purpose, will not fit the Continental eyepieces.

I usually take a well-fitting pill box, cut out a hole in the centre, and fit a piece of thick cover-glass at precisely an angle of 45 degrees.

Third. Nelson's New Method. (*Journal of the Quekett Microscopical Club*, 1913, p. 239). " There is no other method of measuring the power of the microscope that I know of that will give results so near the truth as this, except the troublesome one I have used, and there is none other so easy, simple, and convenient."—*Oxley*.

I quote Mr. Nelson's words :—

" First, it is necessary to determine the ' constant ' of the eyepiece with a given tube-length. This is easily done, and when done it should be recorded, or better still, engraved on the eyepiece tube. To find the ' constant ' of an eyepiece with a given tube-length, first determine the combined magnifying power of that eyepiece on the given tube-length with any objective, say, one of medium power, such as a ⅔-in. or ½-in. or $\frac{4}{10}$-in. focus.

" Secondly, measure the exact diameter of the field by means of the stage micrometer. The product of these two quantities is the constant of that eyepiece.

" Example. Objective ⅓-in., eyepiece compensating × 8, measured magnifying power 280 diameters, measured field 0·023 in. Product is 6·44, which is the constant of that eyepiece for 170 mm. tube.

" The power of any other objective with this eyepiece and any tube-length can be determined by merely measuring the diameter of its field by the stage micrometer ; for the magnifying power will obviously be the eyepiece constant divided by the diameter of the field. Thus, the problem of measuring the combined magnifying power is brought down to the bed-rock of simplicity.

" No camera, no regulation of illumination, no ten inches to measure ; in brief, nothing to do but to count the number of divisions of the stage micrometer in a diameter of the field, and then divide this into the eyepiece constant.

" Example 1. With the same × 8 compensating eyepiece and 170 mm. tube, a ¼-in. objective gave a diameter of field of 0·0165 in. The magnifying power is, therefore, $\frac{6{\cdot}44}{0{\cdot}0165} = 390$.

" Example 2. With the same × 8 compensating eyepiece and 170 mm. tube, a ½-in. objective gave a diameter of field of 0·185. The magnifying power, therefore, is $\frac{6{\cdot}44}{0{\cdot}185} = 35$.

" The determination of the 'constant' is scarcely any more trouble than the measurement of the magnifying power of one objective, and when once found, need not be determined again; it would, indeed, be most helpful if manufacturers would measure these constants and engrave them upon the tubes of their eyepieces.

" Obviously the diameter of the field can be measured while the microscope work in hand is being carried on, for it disturbs neither the microscope nor its adjustments.

" This method has been tested with thirty-three object glasses, ranging from a 3-in. to a $\frac{1}{12}$ of 1·4 N.A., by fourteen different makers and with various eyepieces on three different microscopes with different tube-lengths, and it has been found correct.

" Further experience has shown that, in determining the 'constant,' it is better to measure the magnifying power by direct projection on to a scale, without the intervention of any camera lucida or drawing instrument; the position of the Ramsden's disc, from which the 10-in. projection distance is measured, is easily found by means of a piece of ground glass.

" An excellent scale for the measurement of low powers is a Lufkin 3-in., No. 2,111, price 1*s*."

As Mr. Grundy points out, " There is another easy way in which information given by the 'eyepiece constant' may be used for determining the total magnification for any tube-length.

" Suppose, for example, the eyepiece constant has been obtained with a given objective, eyepiece, and tube-length, a record being made; then it is only necessary to work a very simple proportion sum to determine, at any time, the total approximate magnification with any tube-length. All other conditions being the same, the total magnification will be proportional to the tube-length used.

" It is also worth mentioning that the diameter of the field may be measured in millimetres, instead of inches, if millimetres are used when determining the value of the eyepiece constant."

To Determine the Magnifying Power of an Objective.

Remove the eyepiece, incline the microscope to the horizontal position, place on the stage the stage micrometer, and project an image of this on to a screen 60 in. from the back lens of the objective. Divide the result by six, and the quotient will give the initial magnifying power of the objective at 10 in. to a very near approximation.

The **multiplying power** of the **eyepiece alone** can be determined by dividing the combined power of the objective and eyepiece by the initial power of the objective. Note must be made of the tube-length when the combined power is measured.

Another method of determining the magnifying power of the eyepiece has been given, when the oculars were described (see p. 20).

The Camera Lucida.

There are several forms of these on the market, from the simple Beale's neutral-tint reflector to the more complicated camera lucida of Abbe.

Nelson says: "I find the Abbe camera very difficult to use. Ashe's camera is the simplest, and is easy to use; it is fool-proof. Beale's neutral-tint possesses the disadvantage of reversing the image seen with it, whilst both the camera of Abbe and Ashe do not. I often use Amici's, but find Wollaston's camera very difficult to use, although some prefer it."

For the determination of the combined magnifying power of the microscope Beale's reflector is practically the only one that can be used, but for actual drawing I find by far the best and the easiest to use is the large form of the Abbe-Zeiss camera lucida.

Zeiss and the other manufacturers publish full instructions on the method of using it, and there are only one or two points to which I would draw attention.

A matter of great importance in using the latter model is the vertical and lateral position of the camera on the eyepiece. The camera should be fixed at such an elevation that the eye circle or Ramsden disc coincides with the aperture of the drawing prism. The position of the Ramsden disc can be determined by holding a piece of ground glass or even a cigarette paper over the eyepiece, and moving this up and down till the smallest and most brilliant spot of light is seen.

If the camera be properly adjusted as regards the correct elevation there should be no lateral displacement of the bright Ramsden disc relative to the prism when the head is moved to and fro. Next, the camera must be centred.

" For this purpose it should be seen whether the image of the free objective aperture produced by the eyepiece is exactly concentric with the small aperture left in the silvered surface of the prism. This image appears as a small luminous disc (the Ramsden circle) hanging over the eyepiece.

" In order to judge this correctly the diaphragm of the illuminating apparatus should be opened or closed to such an extent as to render the eye circle equal in size to the unsilvered spot on the prism. When the apparatus is properly centred, the field of the eyepiece should appear to the eye as a clear circle, or, at least, a circle evenly diminished at its periphery by a concentric border of a reddish brown."—*Zeiss.*

The drawing surface must be parallel with the stage, and in order to avoid any distortion in the drawing, the mirror must be inclined at an angle of 45 degrees.

As I found it much easier to work with the microscope inclined to a comfortable position, I have made a very small drawing block, which is shaped like a wedge, and which measures 4 in. wide, 8 in. long, and at one end 4 in. deep, whilst the other end is quite thin.

A small piece of wood measuring about 1 in. in width extends from the inner side of the wedge for about 3 in. This fits under the tailpiece of the microscope, and not only keeps the drawing surface perfectly steady, but ensures that the microscope is inclined in such a position that the drawing surface is parallel with the stage.

I have drawn up a long table of magnifications, with different eyepieces, and at somewhat different tube-lengths, which range from 80 to 4,000 diameters.

Zeiss states that the Huyghenian eyepieces 2, 3, and 4 and the compensating oculars 4 and 6 can be used, but I also find it possible to use comp. oc. 8 and 18.

As to the method of using a camera lucida, whatever be its type, there is only one rule, *i.e.*, the brightness of the drawing surface and that of the microscopic image must be regulated to the requirements of every individual.

Some workers use a feeble light in the image and a strong light on the paper, whilst others require exactly the reverse.

The light on the paper and in the microscope may, to some extent, be regulated by the wonderfully-made darkened glasses fitted to the Zeiss instrument or, as I prefer, by using different darkened glasses in front of the lamp. The surface of the paper may be made brighter or darker by moving a small paraffin lamp towards or away from it.

I have worked for long periods at the camera lucida, and, no doubt, there is knack in it, which one gets by practice, but I have always found it much more trying than observational work, however critical.

To measure the size of an object, such as a trypanosome, from a camera lucida drawing, span the latter with a fine pair of dividers, the points of which are placed a short distance apart, say, 5 mm. The measurement of the drawing in millimetres multiplied by 1,000 and divided by the magnification of the drawing gives the size of the object in μ.

The Care of the Eyes.

We have pointed out that the use of daylight is totally unsuited for critical work, and it is infinitely more tiring than work in a darkened room by lamplight.

All naked lights in the room should be avoided, as obviously the retina cannot remain highly sensitive if it be exposed from time to time to the glare of brilliant electric or other lights.

When I am at work I have sufficient electric light over the shoulder and at the back to enable me to read the "log." of the specimen and to make notes, but when I want to resolve some very delicate structure in the object, I turn out the electric light and wait for a little time in the dark till the retina becomes more sensitive.

As Mr. Eliot-Merlin says : " I find that a darkened room and a well rested eye are essential for all delicate objects. I first get all the adjustments right and then give my eye a long rest. In examining difficult, faintly-stained structures the eye becomes quickly fatigued and requires frequent rests. Straining and long continued looking is fatal to success."

The other great microscopist, Mr. Nelson, when working at concert pitch will not smoke, and will not see any bright light for a week; at the end of that time he finds his eye much more sensitive to delicate images.

It is pitiable to see men working with the unshaded microscope lamp directly in front of them, and no wonder they so soon complain of eyestrain and headache.

One of the most important parts of my outfit is a piece of cardboard about 8 in. long and 3½ in. wide, with a square hole at about the level of the flame. This is placed in front of the lamp and entirely shades the eyes from any light except that coming directly up the microscope tube. I should never think of using my instrument for a minute without such a shade.

It is well seen in the photograph of the microscope on its board; the figures seen on it are the tables of eyepiece micrometer equivalents, etc.

Excessive light in the microscope is very trying, and the use of a blue glass screen, such as I have previously mentioned, very considerably softens the light and removes the objectionable yellowness of the flame, but does not impair the recognition of the stains of the preparation.

The unused eye should be kept open, this is purely a matter of practice. Some observers use the right eye, some the left, others the right or left indiscriminately.

"The effect of years of work with optical instruments on those possessed with strong normal vision seems to be an increase in the defining perception, accompanied with a decrease of the perception of brightness. Those accustomed to use one particular eye with microscopical work and who have done much work would, if they looked at, say, the moon with that eye, see more detail in it than if the other eye were used; at the same time, it would not appear as bright."—*Carpenter.*

Photomicrography.

It may seem somewhat out of place for me to discuss photomicrography, as so many excellent treatises on the subject are now available, but I venture to think that some of my experiences may be of interest and, possibly, of help to those who, like myself, are somewhat new to this subject.

Unfortunately, at the start I had no knowledge of ordinary photography, but when I managed at the first attempt to secure a very fair picture of a particular trypanosome at a magnification of 1,000 diameters, I was impressed with the comparative simplicity of this kind of photography. Since then I have found that it is quite possible to take several photographs on one quarter plate, which is, so far as I know, somewhat of a novelty.

The very first essential in photomicrography is to have as perfect a microscopic image as possible, because the sensitive plate merely receives what is presented to it, and, unlike the eye, has no power of accommodation for different depths of focus.

Mr. Nelson in his presidential address at the Quekett Microscopic Club said: "With some the camera evidently appears to be the end all, and the be all, of the apparatus, but really the camera is the most unimportant part of the whole apparatus, for it can be entirely dispensed with; the late Dr. Woodward, for example, did not use one. The rule is, never mind where you project your image, but wherever it happens to come, there place your plate. The things of primal importance are the obtaining of a visually critical image, and the correct projection of that image, but the exact position of that image has no importance."

The Photomicrographic Camera. Mr. Nelson suggested that a vertical camera would suit my wants better than a horizontal one, and he kindly designed one for me. My friend, Mr. William Harding, slightly modified this and made it for me. The accompanying photograph will give a better idea of its construction than any verbal description, and I will only give the dimensions of the camera. The table, on which the microscope on its board rests, is 17 in. long by 15 in. wide. It is on three strong legs, the feet of which are fitted with rubber and felt. Its surface is 22 in. from the floor. Over this is a box 14 in. high, 12 in. wide and 9¼ in. deep, fitted with a door. It is provided with four legs 11 in. long, which are securely fixed to the table. (Plate III.)

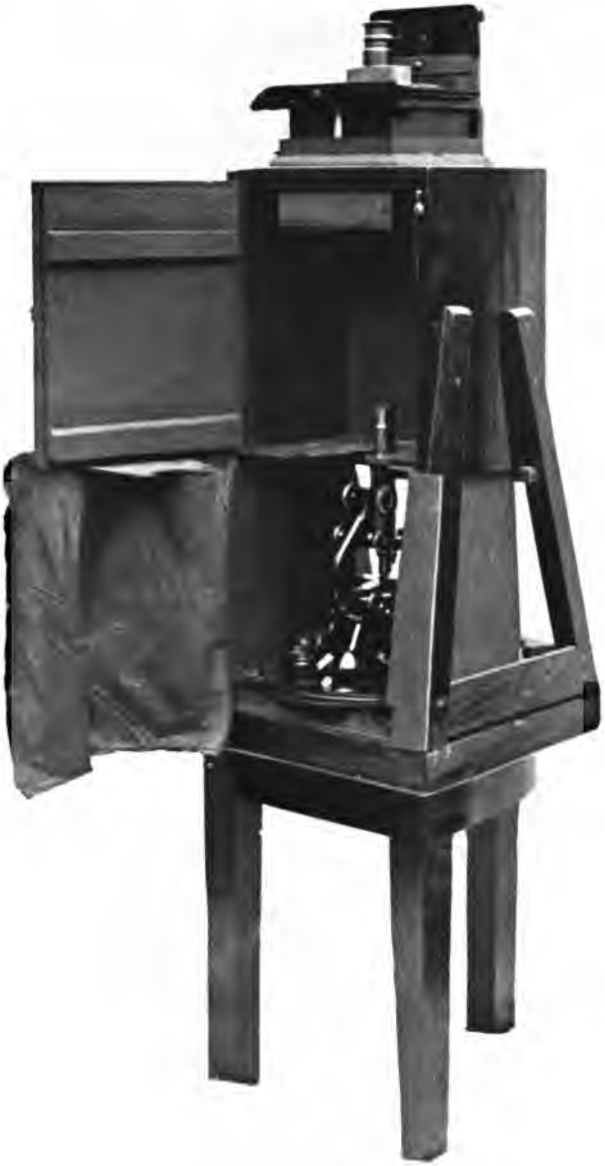
On the top of the box is an old enlarging camera, 7 by 7 in., which has 3 in. of extension marked by a metric scale. The distance from the floor to the surface on which the plate carrier rests is 4 ft. 1½ in., or when the camera is extended 4 ft. 4½ in.

In order to keep the heat of the lamp from the microscope I have fitted a piece of carboard about ¼ in. thick, curved in the same way as the glass globe, which fits in the groove in the microscope board ordinarily used for the globe. It is provided with a small hole opposite the mirror. Another very thick piece of cardboard, also with a corresponding hole, runs smoothly in a groove between the curved cardboard and the lamp. This not only keeps off the heat, but acts as a shutter and is used for making exposures, a movement of about 2 in. being sufficient for this purpose. Neither of these is shown in the photograph. It will, however, be seen from the latter that when the door of the camera is closed the light from the microscope is almost completely shut off from the camera. To ensure this, a thick disc of black velvet is fitted on the lower end of the draw-tube of the microscope.

Some of the text-books lead one to suppose that the light coming up the tube of the microscope passes in a straight line to the plate, but this is not the case, for much light is thrown on to the inner surface of the camera, and reflected in all directions. Such commonly takes place in the smaller forms of the camera bellows. It can be obviated by having the body of the camera large, and by the intervention of diaphragms with small holes with knife edges. Two such are fitted in the top camera.

The same thing must also be attended to in the microscope, not necessarily by enlarging the internal diameter of the body of the microscope, as is usually done, but by seeing that there is a proper stop in the draw-tube, as I have mentioned elsewhere. To make sure doubly sure, I have an iris diaphragm fitted to my compensating ocular 8, and this can be closed down just sufficiently small so that it does not interfere with the picture. In the case

PLATE III.



THE PHOTOMICROGRAPHIC CAMERA.

(*Facing p.* 86.)

of the other oculars, I fit a small piece of cork with a hole in the centre at the lower end of the eyepiece, or a piece of card or metal with a similar hole, about 6 mm. in diameter, fits in the tube of the eyepiece and rests on the lower or field lens. These are made perfectly black, and they have another great advantage beyond that of stopping any internal reflection, in that they ensure the object being placed in the centre of the field. The **plate holder,** or dark slide, is the ordinary double wooden variety.

The **microscope** has needed no alteration beyond the velvet disc which fits over the shoulder of the microscope, at the lower end of the draw-tube. It is, however, of the greatest importance to see that the draw-tube has a stop about 2½ in. down, as previously described (see p. 3).

Compensating eyepieces are used to obtain the necessary magnification. I only use the objective alone in very rare cases.

Focussing Glass.—In all my work, which is usually at a magnification of 1,000 to 2,000 diameters, I rely entirely on focussing the image with a focussing glass, and after many trials I found that made by Watson suited my purpose the best. This is focussed once for all on the clear glass plate, and is fixed by screwing it up tightly, and it is not afterwards altered.

I never use the ground glass focussing screen except for very low magnifications, and then only to see the extent of the image. I do not even focus the image on the clear glass screen, but focus an aerial image without the intervention of any screen. This is accomplished by fixing the focussing glass in a piece of wood, 6 in. by 1¾ in., with a hole in the centre not quite 1½ in. in diameter. The focussing glass is fixed at the same level or register as the film surface of the plate.

Colour Screens.—As practically the whole of my work is with films stained with Giemsa or Leishman's stains, I only use one screen, viz., Wratten's B3 or No. 56. This is of a greenish-yellow colour, and it is a lighter and more luminous edition of their B (green) and G (yellow) screens. The former, B3, apparently gives just as good results with the paraffin lamp as the latter, and does not require such a long exposure.

Plates.—I have tried the following quarter plates:—Gem isochromatic, Edward's medium isochromatic, Ilford's rapid chromatic, and Imperial N.F. (nonfilter), and as far as I can tell there is very little difference between them. Latterly I have kept entirely to the Imperial N.F. plates. I have not tried the panchromatic plates.

To determine the Magnifying Power with the Camera.—Place on the stage of the microscope the stage micrometer, on the top of the camera the clear glass screen, and on the surface of the latter lay the Zeiss measure with the figures reading correctly. (Zeiss' measuring scale on plate glass is a very useful and beautiful measure. The figures are on the under surface in mirror type, so that the divisions may be laid on the surface of objects to be measured, and so easily read off without parallax. It cost pre-war 3*s*.). Over the scale place the focussing glass and focus the image of the lines of the stage micrometer. It is advisable to keep as far as possible to whole numbers.

I have constructed a table from which I can see at a glance what eyepiece used, say, with the $\frac{1}{16}$-in., and what camera extension, will give a magnification of 1,000, 1,500, or 2,000 diameters precisely, for various tube-lengths. Similar tables have been constructed for the other oil immersion lenses, the 8 mm., and $\frac{2}{3}$ in. at various tube-lengths.

The following calculations may be of help in photomicrographs and camera lucida drawings.

1. To find the size of the object.

Measurement in mm. of image in photo $\times \dfrac{1{,}000}{\text{magnification}}$ = size of object in μ.

Example: Image = 8 mm. and the magnification × 400.

$$8 \times \frac{1{,}000}{400} = 20\,\mu \text{ the size of object.}$$

2. To find the size of the image in photo.

Size of object in $\mu \times \dfrac{\text{magnification}}{1{,}000}$ = size of image in mm.

Example: Object = 20 μ magnification = 400.

$$20 \times \frac{400}{1{,}000} = 8 \text{ mm. the size of image.}$$

3. To find the magnification.

$$\frac{\text{Size of image in mm.}}{\text{Size of object in }\mu.} \times 1{,}000 = \text{magnification.}$$

Example: Size of image = 8 mm. Size of object = 20 μ.

$$\frac{8}{20} \times 1{,}000 = 400 \text{ magnification.}$$

It is very difficult to describe on paper my method for taking a number of photographs on one quarter plate, but the principle is somewhat as follows: The whole exposed part of a quarter plate is about 100 by 77 mm., and supposing I want to take sixteen separate photographs on that area, I cut a small oblong hole 25 by 19 mm. out of a piece of zinc, and use that as a mask. By a home-made device sixteen areas of the sensitive film are exposed over this open area. I found cutting the masks a very difficult performance, but the results are quite satisfactory. Some such apparatus made in metal by a skilled mechanic would, I am convinced, prove invaluable to many workers.

By my system I can take 1 : 2 : 4 : 9 : 12 : or 16 photographs on a quarter plate under the same or under totally different conditions as regards magnification, etc.

Of course, if sixteen exposures are made of different slides, different subjects and different staining intensity, even at the same magnification, the resulting images on the negative will not all have the same density, and the negative will require some negotiating in printing.

Two **focussing screens**, one of clear and the other of finely-ground glass, were made in register with the plate holder; but absolute register is unimportant in photomicrography.

Illumination.—For reasons of convenience I have always used the ordinary microscope lamp with ½-in. wick. Latterly I have used a 1-in. wick, folded in its long axis, so as to form a wick ½-in. in one direction and double the previous thickness. This gives a slightly wider flame image, and probably one of somewhat greater luminosity.

The time of exposure depends on very many factors, and I have found two small pamphlets published by Kodak, Limited (Wratten Division), Kodak House, London, W.C. 2, of the very greatest help. They are " Photomicrography " and " Notes on the Use of Wratten's ' M ' Filters." The former is a pre-war production and the latter a reduced form of it. They can be obtained for a few pence. They contain valuable information on the nature and the use of colour screens, time exposure, etc. The time exposure of a plate varies inversely as the square of the W.A. (working aperture) of the objective, and directly as the square of the magnification, "It is the W.A. (' working aperture ') of the lens and not its N.A. that matters, *e.g.*, if you use a cone of 0·3 N.A. on your ⅓-in. objective, its photographic speed would be the same as that of your Leitz three objective (nominally ⅔) used at full cone."—*Nelson.*

Almost all my work is done with oil immersion lenses of N.A. 1·30, and at magnifications ranging from 500 to 3,000 diameters. I do not use the 8 mm. frequently for photography, and when I do I am a little uncertain as to the time of exposure that would give the best results. When using the oil immersion objectives, particularly a very fine 1/16-in. Leitz, and compensating oculars, I find about one and a half to two minutes the correct time for a magnification of 1,000 diameters with critical illumination, and the edge of the flame of ½-in. wick, and screen B.3.

I practically never use a bull's-eye with these powers, as it destroys, according to all the authorities, the critical nature of the image. My photographs are always taken in the area of the flame image, and if this does not give a large enough field I turn the lamp slightly sideways. If, however, I want a larger photograph with the high, but more particularly the medium and low, powers, I use the small bull's-eye described before, and if that is not sufficiently large, the achromatic bull's-eye.

Assuming that a duration of one, one and a half or two minutes is the correct time of exposure for a magnification of 1,000 diameters, and as the exposure varies as the square of the magnification, the following table should be about correct.

I find that there is a considerable range in the time exposure with the isochromatic plates, and in many cases I cannot tell the difference between one or two minutes' exposure. (See the

Magnification.	Time.		
500	15 sec.	22 sec.	30 sec.
1,000	1 min.	1½ min.	2 min.
1,500	2 min. 15 sec.	3 min. 22 sec.	4½ min.
2,000	4 min.	6 min.	8 min.
2,500	6 min. 15 sec.	9 min. 22 sec.	12½ min.
3,000	9 min.	13½ min.	18 min.

remarks previously made on the effect of the thickness of the slide on time exposure, p. 59.)

Development.—I always develop the plate in total darkness for the following reasons: That my knowledge of ordinary photographic work is very limited, that most of the images are too small to be seen by the light of a dark room lamp, that the quarter plate has so many photographs, and that, lastly, there is a risk of fogging a plate, even with a red light.

I proceed as follows: I put the developing dish in a cardboard box, place the negative in, and pour on the developer in total darkness. I then, after giving the box a shake, put on the cover, and leave it, returning about every minute or so to give it a shake, and at the end of about ten to twelve minutes, at average temperatures, I remove it, wash it in a dish of water and, finally, place it in the hypo solution. This I cover up and leave for about ten minutes, and then wash in running water.

I have only used one developer throughout, that described by Watkins in his little manual on photography. This is:—

Solution A.		*Solution B.*	
Metol	½ dram.	Sodium carbonate crystals	1 oz.
Hydroquinone	1 „	Sodium sulphite	1 „
Pot. metabisulphite	½ „	Water	20 „
Water	20 oz.		

Dissolve in the order named.

For use, equal parts of *A* and *B* are taken.

Printing.—I am told by professional photographers that printing by P.O.P. paper will give all the details in a negative better than by any other means. Partly for this reason, but chiefly from the fact that my negatives contain so many images which are often unequal in their printing density, I only use the P.O.P. process. With this method one sees the pictures as they print, and it is easy to stop out some of them by placing a piece of paper on the glass side of the negative, so that it is quite possible to obtain a print of, say, sixteen photographs of nearly equal tint from a negative in which the images are very unequal. I usually tone the print rather deeply with the gold toning solution.

My actual procedure in taking photographs, say, of *Spirochæta pallida*, stained with Giemsa's stain at a magnification of 1,000 diameters, would be somewhat as follows: See that the eyepieces, mirror, condenser, and glass in the lamp are perfectly clean. Find a suitable spirochæte and place it in the centre of the field, and put the colour screen in position. Centre the condenser, focus the image of the edge of the flame, and determine the exact tube-length necessary. Do not use the bull's-eye if it can possibly be avoided. Suppose the tube-length be 170 mm. from my table of magnification, I find that the $\frac{1}{16}$-in. with compensating ocular 4, and the camera extended to 14 mm., gives precisely the required magnification.

I now lay a small lead weight on each end of the slide, the object of which will be obvious in a moment. The image of the flame and the object are put exactly in the centre of the field, which is a very small circle, as the eyepiece is fitted with a diaphragm.

The microscope is now raised to the vertical, and the screen, to protect it from the heat of the lamp, placed in position.

Everything now being in perfect adjustment, the only question remaining is what cone shall be used. For visual purposes we always work with a three-quarter cone, and, personally, I try as far as possible to use about the same for photography.

If more contrast is required, the use of a somewhat smaller cone is generally advised, but there is always danger in cutting this down.

The microscope board, with everything in adjustment, is placed in the camera, and the door closed.

With the focussing glass on the top of the camera, the image is carefully focussed, and arranged perfectly centrally in the field. If the image is not in the centre, a slight movement of the whole board with the microscope will soon correct it, and this is infinitely easier than moving the mechanical stage.

Still watching the image through the focussing glass, I give the whole apparatus one or two sharp taps, just to make things settle down, and correct, if necessary, the focus.

The focussing glass is removed, the dark slide very gently placed in position and the exposure made by sliding the cardboard screen out about an inch. At the end of one-and-a-half to two minutes, the cardboard screen is gently pushed in, the dark shutter closed, and removed as carefully as possible. Without altering anything the focussing glass is replaced in position, and the image examined.

If the object is still in perfect focus we may rest assured that the image will be as good as the objective will give.

On the other hand, if the image be not in perfect focus, it does not necessarily follow that the result will be a failure, because the change of focus may have taken place after the plate had had its exposure, but in all probability the photograph will not be a success.

This last point brings us to the very important question as to the **effect of vibration in photomicrography.**

I will again quote from Mr. Nelson's presidential address. He says: " While on this subject it might be pointed out that vibration, a spectre which for long has haunted photomicrographers, myself included, is in practice found to be a myth. If there be a microscope in the world more likely to be influenced by vibration than any other, it is Powell's No. 1, for it has a long body and a long bar movement; nevertheless, during all the years it has been in use not a single negative has been lost through vibration, and what is more, I never heard of any one else losing one from that cause.

" This being the case, how much less likely is a crane arm microscope to be affected by vibration? But so powerful has been the influence of the spectre, that it is deemed necessary to have heavy metal beds for these microscopes, for the purpose of strutting up the end of the crane arm. This, together with drawtube and eyepiece clamps, is thought to be the correct design for a photomicrographic instrument. We now come to the funny part of the design in the class of apparatus referred to, for when all this hypercritical care is taken to make the instrument as rigid as a solid block of metal, the wooden base is cut and the whole put on a pivot. Even granting that a pivot is necessary, it is the camera with its base board that should be turned aside, the microscope being left unmoved on a firm trestle, which arrangement was invented some time back, and is still used.

" The vibration scare is in a great measure due to the neglect of the re-examination of the image after the photograph has been taken ; if that had been done it would be found that the loss of the negative was due to an alteration in focus, and not to vibration."

Although in my case the trams are running near the house, I have never noticed any harm done, even at a magnification of 3,000 diameters, but if during an exposure I drop a book near the camera, I shall find that the focus, in nine cases out of ten, will have altered, and the negative will be a bad or an indifferent one.

In the vertically placed microscope, does the focus always drop downwards, or, in other words, will it be necessary to rack the instrument upwards to correct the focus ? No, not necessarily so. Try the following experiment. Remove the microscope from the camera and place it on the table with the tube either vertical or inclined. Focus any object exactly, and remove the hand from the fine adjustment. Tap the table or the microscope board sharply two or three times, watching carefully the image, and in almost all cases it will be seen that the object goes out of focus, but notice that sometimes the focus can be recovered by turning the screw of the fine adjustment upwards, sometimes downwards. One can understand why the object might go out of focus in a downward direction, but why in an upward direction ? It is not a question of heat from the lamp, but in my case, at least, the slide is probably not in actual contact with the surface of the stage when it is first focussed, as it is only held in position by clips at the end and not on the surface of the slide. In order to obviate this I place two little weights of lead, one on either end of the slide, to hold it down.

If I were asked what were the two most common causes of indifferent photomicrographs, I should undoubtedly say, firstly, an imperfect microscopic image due, in most cases, to uncritical adjustment of the visual image in the microscope, or some imperfection in the objective, etc., and secondly, an alteration in the focus taking place during the exposure.

Photomicrographs on Frontispiece.

Upper Plate.

Sixteen photomicrographs of Protozoa pathogenic to man, taken on a quarter plate at a magnification of 1,000 diameters precisely.

1. *Spirochæta pallida*—man.
2. *Leptospira icterohæmorrhagiæ*, spirochæte of infective jaundice, from kidney of naturally infected rat in England. Note the very fine spirals. Compare figs. 19 and 20.
3. *Spirochæta carteri*, spirochæte of relapsing fever (China).
4. *Leishmania donovani*, from spleen of case of *Kala azar*.
5. *Leishmania tropica*, from case of Oriental sore.
6. *Trypanosoma gambiense* in rat, from man with sleeping sickness, showing the long and thin, and the short and thick, forms.
7. *Trypanosoma rhodesiense* in rat, from man. Sleeping sickness of Rhodesia, showing the long and thin, and the posterior nuclear, forms.
8. *Trypanosoma cruzi* in guinea-pig, from man. South American trypanosomiasis, showing variations in size.
9. Malaria fever. Benign tertian, showing a small ring and two half-grown parasites with Schüffner's dots in enlarged red corpuscles.
10. Malaria fever. Benign tertian, segmenting form.
11. Malaria fever. Quartan, half-grown parasite. Note the red cell is smaller in size.
12. Malaria fever. Quartan, segmenting form.

13. Malaria fever. Malignant tertian, small ring parasites with Maurer's dots in the red corpuscles.
14. Malaria fever. Malignant tertian, small ring parasites in almost all the red cells and a segmenting parasite, from the peripheral blood.
15. Malaria crescents, male and female, micro- and macrogametes.
16. Malaria crescent, flagellating microgametocyte.

Lower Plate.

17 and 18. *Spirochæta morsus muris,* the spirochæte of rat-bite fever. Preparation from Dr. Row, Bombay. × 2,500.

19 and 20. *Leptospira icterohæmorrhagiæ,* spirochæte of infectious jaundice. No. 20 from kidney of naturally infected rat in England. No 19 from inoculated guinea-pig, from man. Note the very small spirals. × 3,000.

21. *Trypanosoma evansi,* the trypanosome of Surra. × 1,000.
22. Trypanosome of thrush, large form. × 1,000.
23. *Trypanosoma lewisi,* naturally infected rat, showing also a coiled up trypanosome. × 2,000.
24. Trypanosome of bat (pipistrelle). × 1,000.
25. Halteridia of thrush, two microgametes. × 1,000.
26. Leucocytozoa of thrush, male pale, female dark. Micro- and macrogametes. × 1,000.
27. Cysts of *Entamœba coli,* fresh preparation stained with iodine. Note the nuclei and the variations in size of the cysts. × 400.

Staining and Mounting.

A few notes on the method of staining with Giemsa's stain, to which allusion has been frequently made, and the mounting of film preparations in liquid paraffin, may be of some service.

To clean Slides.—If new, rub them with a paste made by mixing prepared chalk (*creta præparata*) with water, then drop them in water, or allow them to dry and polish with a clean cloth.

If old, remove any balsam, cement, or dried oil with a knife, and after rubbing them with a piece of cloth moistened with spirit, treat as above.

Cover-glasses.—I now prefer to use long oblong, 1¼ in. by $\frac{15}{16}$ in., thin No. 1 covers. To clean, drop them one by one into a wide-mouthed bottle containing strong sulphuric acid, in which they remain all night. Then pour off the acid, thoroughly wash them in running water, then in distilled water, and store in spirit.

To spread Films.—Place a drop of the blood, pus, exudation, etc., on one end of a slide and with the edge of another slide held at an angle of 45 degrees, spread in as thin and uniform a layer as possible. Dry in the air; or better, expose the film side, whilst wet, to the vapour of a solution of iodine in chloroform for ten to sixteen seconds until it is distinctly yellowish, then allow to dry in the air.

To Stain.—Procure Giemsa's stain ready made, or make it as follows: Giemsa's (*azur-eosin*) powder 0·4 grammes; pure glycerine 25 c.c.; pure methylic alcohol 75 c.c.

For use, take one part of either of the above solutions and add about two parts of methylic alcohol; filter this into a small dropping-bottle fitted with a ground-in pipette stopper and rubber teat. Drop a few drops of this on the air-dried film and allow it to remain about half a minute, then drop from a drop-bottle about five to ten times as much distilled water, and place the slide with the stain and water, film slide downwards, on a curved piece of glass.

(Procure a piece of show-case plate glass cut to about 8 by 4 in., and use the concave surface of this as a staining dish. It will hold seven to eight slides placed side by side.) Allow the stain to act for any time from five minutes to twenty-four hours, then wash with distilled water, and dry in the air.

To Mount.—All aniline stains fade in the course of time when mounted in Canada balsam, even if this be Grubler's neutral Canada balsam in xylol. The colour keeps much better, but even then is not absolutely permanent, if the preparation be mounted in parolein or liquid paraffin, as I pointed out in an article in the *Lancet*, April 1st, 1911 ("The Fading of Aniline-stained Microscopical Preparations"), and which Giemsa later, possibly independently, advised in 1913.

Clean a cover-glass, gently warm over the spirit lamp to dry off any moisture, and place a small drop of liquid paraffin or parolein on the surface. Then dry the stained preparation in the same way, and gently lower it, film side downwards, on to the drop of oil on the cover. Press out any excess of oil, and dry the margins as well as possible, and then paint round the edges with Grubler's asphalt cement, which is probably neutral in reaction.

Address of Microscope Makers, Agents, etc.

Baker, Charles, 244, High Holborn, London, W.C. 1.

Beck, R. and J., 68, Cornhill, London, E.C. 3.

Flatters and Garnett, 309, Oxford Road, Manchester.

Hawksley & Son, 83, Wigmore Street, London, W. Agents for Koristka, and Spencer Lens Co., U.S.A.

Kodak, Ltd. (Wratten Division), Kodak House, London, W.C. 2 (see page 103).

Swift & Son, 81, Tottenham Court Road, London, W. 1.

Watson & Sons, 313, High Holborn, London, W.C. 1 (see page 102).

Leitz—Agents: Ogilvy & Co., 18, Bloomsbury Square, London, W.C. 2 (see page 101).

Bausch and Lomb Optical Co., 37 and 38, Hatton Garden, E.C.

Zeiss—Agent: J. W. Atha, 8, Southampton Row, London, W.C. 2 (see page 104).

The British Drug House, Ltd., 22 to 30, Graham Street, City Road, London, N., for aniline dyes and microscopic re-agents.

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